

THREE NEW
LIFE-HISTORIES

in the

**Trematode Family
Strigeidae**

Together with a

Description of a New Species

With Six Plates

and

Three Text Figures

—by—

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submitted in partial fulfillment of the requirements for the
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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE
(HOLOSTOMIDAE). XXI. LIFE-CYCLE AND DESCRIPTION OF THE CERCARIA OF *COTYLURUS*
MICHIGANENSIS (LARUE) *

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During the summer and autumn of 1926 and 1927 the life-cycle of a trematode of the family Strigeidae was experimentally followed through the stages of tetracotyle, adult, egg, miracidium, and cercaria. The tetracotyles of this life-cycle, found on and around the hearts of common suckers, *Catostomus commersonii* (Lacépède), were developed into adults in the bursa Fabricii of the herring gull, *Larus argentatus* (Pont.). Adults of the same species obtained from naturally infested herring gulls have been described in a preliminary report by LaRue (1927: 226) under the name of *Strigea michiganensis* LaRue. Since that time Szidat (1929: 612-764) has divided the genus *Strigea* into seven genera. According to Szidat's classification those species of the former genus *Strigea* whose vitellaria are confined to the hindbody and which have a tongue-shaped process projecting into the bursa copulatrix from its antero-ventral wall should be placed in the genus *Cotylurus* Szidat 1929. The adults of the present species possess both of these characters and should therefore now be named *Cotylurus michiganensis* (LaRue).

The tetracotyle of this species was carefully described by Hughes (1928: 415-419) as *Tetracotyle communis* Hughes, a name which falls as a synonym of the earlier name proposed for the adult stage. Hughes found these tetracotyles on and around the hearts of several species of fish, among them the common suckers of Douglas Lake. The tetracotyles employed in my feeding experiments were morphologically identical with those described by Hughes and were also found on the hearts of common suckers of Douglas Lake during the same summer (1927) that Hughes collected his material.

The herring gulls used in these experiments were obtained from their nesting grounds, Goose Island in the Straits of Mackinac, when about two weeks old. They were kept in wire-screened pens on sand and, both before and during the experiments, they were fed cooked fish varied with occasional pieces of fresh beef. The water given them was

* Contribution from the Biological Laboratory of Calvin College and from the Biological Station and the Department of Zoology, University of Michigan. This is the XXI of a series of studies on the family Strigeidae by Professor George R. LaRue, his students and associates.

pumped from a well. In this way the young gulls were protected against strigeid infestations for a week or two before the experimental feedings began. By the time the gulls were three or four weeks old they were large and strong enough to bear heavy infestations. Owing to the parasites which these gulls had obtained while they were still on their nesting grounds a few strigeid eggs were generally found in their feces before the experiments began.

* After their removal from the fish the hearts of the common suckers with their tetracotyles were placed in watch glasses and fed to seven young gulls at the rate of about one to three hearts a day for each bird. At the end of two weeks of such experimental feeding two of the gulls died. The other five gulls were killed after being experimentally fed for three to four weeks. The emaciation and paralysis of these gulls, undoubtedly due to their infestations, were symptoms similar to those reported and explained by LaRue (1927:226) for his birds. Upon examination it was found that the bursa Fabricii of every one of my gulls was closely packed with large numbers of mature and immature *Cotylurus*. Other young gulls, not fed materials known to contain tetracotyles, either had only a few *Cotylurus* in their bursae or none at all. The number of *Cotylurus* found in the bursa in each of the seven purposive feeding experiments was so unusually large and included such a gradation of stages of development that there is no doubt that the *Cotylurus* found in the bursae of the experimental gulls developed from the tetracotyles encysted on and around the hearts of the common suckers.

A critical examination of these bursal parasites indicated that they all belonged to one species. Professor G. R. LaRue who had made a careful study of the adults of this species, informed me that he also had found only one species in the bursa Fabricii of his gulls from the same region. Since my adults discussed in the present paper closely resemble the parasites described by LaRue (1927:226) it is believed that all of my specimens obtained from the bursae of the experimentally fed gulls represent *Cotylurus michiganensis* (LaRue).

The eggs used in this study were obtained by slitting open the bursae of the experimentally fed gulls and shaking them in water. The shaking released a large number of eggs and massive concretions containing hundreds of eggs. The concretions were broken up in order to free the eggs. In this way thousands of eggs were quickly and easily collected. The collections of eggs, separated from all other matter, were kept in well-water in labelled watch glasses until the miracidia appeared.

Since only one species of adults was found in the bursae of my gulls, it is believed that the eggs obtained from these bursae also represented only one species. Because the opening of the bursa into the

intestine is small it is improbable that the eggs of parasites living in the intestine would enter the bursa. This is particularly true of the experimental gull whose bursa supplied the eggs that yielded the later developmental stages of the present life-cycle. My record shows that the intestine of this gull contained only two specimens of *Diplostomum* and no other strigeids while the number of *Cotylurus* in its bursa was large. Moreover, only one kind of cercaria was finally obtained from these eggs and this cercaria could readily be distinguished from the cercaria of an intestinal *Diplostomum* of the herring gull. The evidence for this statement will be presented later in connection with the description of the cercaria of *Diplostomum flexicauda* (Cort and Brooks).

Nineteen days after the eggs were collected the miracidia hatched. A study of these miracidia alive revealed that they resembled those of *Diplostomum flexicauda* (unpublished researches) so closely that they are not described here. With the miracidia of *C. michiganensis* five infestation experiments were made involving the following kinds of snails: *Planorbis trivolvis* Say, *Lymnea stagnalis perampla* Walker, *L. lanceata* Gould, *L. emarginata angulata* Sowerby, *Physa parkeri* "Currier" De Camp, *Physa gyrina* Say, and *Planorbis campanulatus* Say. In each experiment three or four snails of different species were exposed together in one watch glass to eight or ten miracidia. Inadvertently a few eggs may also have been transferred with the miracidia to the watch glasses containing the snails. Although the miracidia were carefully watched the actual penetration of a miracidium into a snail was not observed. Thereafter the contents of each watch glass were kept in a labelled bottle with filtered water. The snails were fed lettuce and the water in the bottles was changed every second day.

One of the snails, *L. emarginata angulata* Sowerby, only about half grown, which had been exposed to the miracidia, and possibly also to eggs, on September 4, 1927, produced large numbers of cercariae which were first observed on October 14, 1927, forty days after exposure to the miracidia. This snail was one of a collection of snails obtained from Douglas Lake and kept in an aquarium in the laboratory at least three weeks before it was exposed to the above mentioned miracidia. It could not have become infested in the aquarium since it was kept in well-water and was fed lettuce grown far from the lake. The possibility that this snail was already infested when it was collected is not altogether excluded, but after an examination of the facts presented below appears to be improbable. Of the remaining snails of the collection including the same and other species not one produced the present kind of cercariae. Moreover, the cercariae first appeared forty days after the snail was experimentally exposed to the miracidia and more than sixty days after it was collected from Douglas Lake. Experiments by Mathias (1925:

55) have shown that a period of six weeks was sufficient for the development of the cercaria of *Strigea tarda* (Steenstr.) from its miracidium. Since the weather was unusually warm during the month of September, 1927, there is no reason to believe that a longer period than forty days would be required in this case. This argument is also supported by three other experiments (described in a later paper) which I performed at the same time and in which the cercariae of *Diplostomum flexicauda* (Cort and Brooks) were developed from their miracidia in forty-five days, the snails in these experiments being kept under the same conditions as the snail producing the cercaria of *Cotylurus michiganensis*. From these considerations it seems entirely improbable that the development of the present cercaria from its miracidium required more than sixty days, which would be the case if the snail had been infested before it was collected from Douglas Lake.

If the foregoing discussion of this life-history is correct, the species of cercaria resulting from my infestation experiments is the cercaria of *Cotylurus michiganensis* (LaRue). This cercaria represents a stage of development of the first American life-history in the family Strigeidae which has been experimentally traced through two vertebrate hosts. No attempts were made to infest a common sucker with this cercaria since this fish was not readily obtainable at that time. Consequently, to remove all doubt it seemed desirable to repeat the experiments which connect the various stages of this life-history.

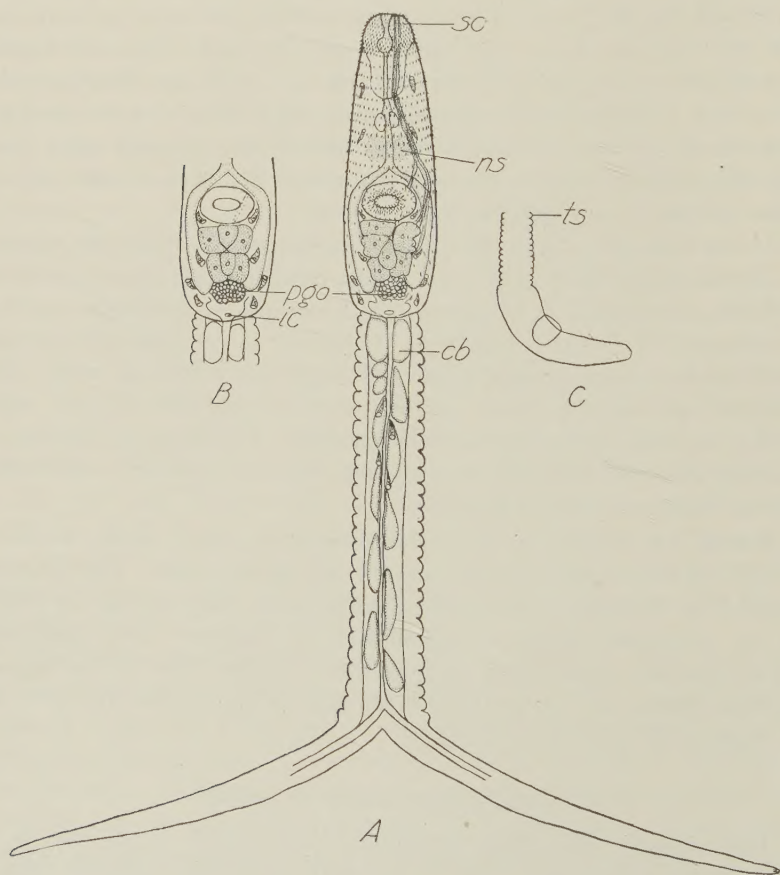
During the summer of 1928 infestation experiments, similar to those of 1927, yielded results similar in every way to the latter. The cercaria obtained in 1928 was specifically identical with that obtained in 1927, but no additional details of life-history or structure were observed. These repeated experiments support the contention that all the above reported stages of *Cotylurus michiganensis* belong to the life-cycle of the cercaria which is here described.

Cercaria michiganensis (LA RUE)

This cercaria, like other cercariae of the family Strigeidae, is a furcocercous form with a pharynx and with an excretory bladder which extends into the tail-stem (Text fig. A). While at rest it assumes a characteristic position (Fig. C). With furcae widely outspread it hangs in the water with the body downward and bent ventrally, the region of greatest bending being near the acetabulum.

This cercaria is small, its total length being about 0.8 mm. when measured alive and free from coverglass pressure. In this condition the body measures 0.22 mm. in length; the tail-stem, 0.30 mm., and the furcae of the tail, about 0.28 mm. Measurements of four specimens, fixed in hot 5% formalin, were 0.22-0.24 mm. for body length, 0.32-0.36

mm. for length of the tail-stem, and 0.24-0.27 mm. for length of the furcae. The body is widest immediately behind the acetabulum where it measures 0.06 mm. The oral sucker is pear-shaped, narrowed posteriorly, and about 0.065 mm. long. The acetabulum, circular in ventral view, is 0.035 mm. in diameter while its dorsoventral dimension is approximately two-thirds of the thickness of the body.



Figs. A, B, C.—*Cb*, caudal body; *ic*, island of Cort; *ns*, nervous system; *pgo*, primordium of genital glands; *sc*, spinous cap; *ts*, tail-stem.

Numerous small spines are arranged over the surface of the body in transversely encircling rows. They are largest, most numerous, and most closely set at the anterior part of the body and more sparsely distributed behind the acetabulum so that their arrangement in transverse rows is less evident there. At the anterior part of the body the first 15 or 16 rows of spines are so close together that they seem to form a

distinct spiny cap, in which the spines have a quincunx arrangement. Six rows of closely set spines surround the opening of the acetabulum while one row is found around its outer margin.

The digestive system is well developed. The dorsal side of the mouth has a projection where the ducts of the penetration glands open. The narrow prepharynx, 0.05 mm. long, is widest immediately in front of the pharynx which is few-celled and spherical, 0.02 mm. in diameter. The esophagus extends more than half way from the pharynx to the acetabulum. Its diameter exceeds that of the prepharynx. The digestive ceca diverge around the acetabulum and extend along the dorsal side of the body almost to its posterior end. Posterior to the acetabulum they are dilated. They contain a uniformly hyaline material.

Three pairs of unicellular penetration glands with coarsely granular contents are arranged typically behind the acetabulum in three longitudinal rows of two cells each as shown in figure B, but their position varies with the contraction states of the animal, one of which is shown in figure A. During contraction of the body the anterior group of these glands may move forward dorsally over the acetabulum. The ducts of the lateral glands extend along the sides of the acetabulum ventral to the digestive ceca, while the ducts of the median glands extend dorsally over the acetabulum. Farther forward these ducts extend together along the sides of the pharynx and through the oral sucker to the anterior end of the body. When stained with thionin it becomes evident that the ducts retain their individuality throughout their course. These ducts are filled with granular contents like those of the glands.

Immediately behind the penetration glands is found a group of cells generally considered to be the primordium of the genital organs. These cells stain deep blue with thionin. According to Szidat (1923: 310) the primordium of the genital glands arises *de novo* during the development of the tetracotyle from the cercaria. Szidat's statement raises the question whether the so-called primordium of the genital organs of strigeid cercariae has been correctly named.

The main part of the excretory system consists of a bilobed bladder surrounding an island of Cort. From this bladder two lateral vessels extend anteriorly, one along each side of the body, while a third branch extends posteriorly in the median line of the tail-stem and divides at the posterior end of the tail-stem into two secondary branches each of which extends into a tail-furca. Although these secondary branches have not been traced to their openings, it is believed that each opens near the middle of the dorsal edge of its furca. The lateral vessels have not been traced to the flame cells in which they finally terminate. Eight pairs of flame cells, six in the body and two in the tail-stem, were found in the positions indicated in figure A.

Usually six pairs of caudal bodies containing a pale greenish-yellow fluid substance were found in the tail-stem. Sometimes one of these bodies appeared to be divided into two or more smaller bodies as shown in the figure. Similar bodies of smaller size were also found distributed along the furcae of the tail.

SUMMARY

1. The life-cycle of *Cotylurus michiganensis* (LaRue) was traced by means of infestation experiments.

2. Tetracotyles (*Tetracotyle communis* Hughes 1928) found around the hearts of *Catostomus commersonii*, were fed to young herring gulls, *Larus argentatus*. As a result large numbers of adult *Cotylurus michiganensis* (LaRue) were developed in the bursae Fabricii of the herring gulls.

3. Eggs collected from these bursae produced miracidia in somewhat less than three weeks. Of snails exposed to the miracidia, only two specimens of *L. emarginata angulata* became infested. These two first produced cercariae about forty days after exposure to miracidia.

4. These cercariae differ from other known cercariae by a complex of characters which were fully described.

This study was conducted under the direction of Professor George R. LaRue of the Department of Zoology and Director of the Biological Station, University of Michigan. His kindly interest and helpful suggestions are gratefully acknowledged. I am also indebted to Miss Mina L. Winslow of the Museum of Zoology for identification of the snails.

LITERATURE CITED

- Cort, W. W. 1917.—Homologies of the excretory system of the forked-tailed cercariae. Jour. Parasit., 4: 48-57, 2 text-figs.
- Hughes, R. C. 1928.—Studies on the trematode family Strideidae (Holostomidae). No. XIII. Three new species of tetracotyle. Trans. Amer. Microsc. Soc., 47: 414-433, 2 plates.
- LaRue, G. R. 1927.—A new species of Strigea from the herring gull, *Larus argentatus* (Pont.), with remarks on the function of the holdfast organ. Jour. Parasit., 13: 226.
- Mathias, P. 1925.—Recherches expérimentales sur le cycle évolutif de quelques trématodes. Bull. Biol. France Belge, 59: 1-123, 4 pl.
- Szidat, L. 1923.—Beiträge zur Entwicklungsgeschichte der Holostomiden. I. Zool. Anz., 58: 299-314, 9 text-figs.
- 1929.—Beiträge zur Kenntnis der Gattung Strigea (Abildg.). Ztschr. Parasitenk., 1: 612-764, 70 text-figs.

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STUDIES ON THE TREMATODE FAMILY
STRIGEIDAE (HOLOSTOMIDAE)
NO. XXII: *COTYLURUS FLABELLIFORMIS*
(FAUST) AND ITS LIFE-HISTORY *

JOHN P. VAN HAITSMAN

PART I. THE ADULT

THE trematode species of the family Strigeidae here described as *Cotylurus flabelliformis* (Faust) was first found in wild and domestic ducks of Michigan during the summer of 1925. Adult parasites of this species have a distinct cup-shaped forebody (Figs. 11 and 12), inside of which there are lateral sucking cups, characters which place these parasites in the subfamily Strigeinae Railliet, 1919. This species is further characterized by a tongue-shaped process projecting from the antero-ventral wall of the bursa copulatrix and by the distribution of its vitellaria, which are confined to the hindbody. These two characters determine the place of this species in the genus *Cotylurus* Szidat, 1929.

No species of *Cotylurus* has thus far been described from ducks of North America, although Rettger (1897, pp. 224-225) has reported the finding of holostomes in ducks of Indiana. The material which I secured from American ducks has been carefully compared with two descriptions of similar parasites of Europe: that of *Strigea tarda* by Mathias (1925, pp. 1-65) and of *Cotylurus cornutus* by Szidat (1929, pp. 736-739). According to Szidat, *S. tarda* is an objective synonym of *C. cornutus*. Sufficient differences in size and structure were found to justify the description of my material as a new species. By means of infection experiments, to be de-

* Contribution from the Biological Laboratory of Calvin College and from the Biological Station and the Zoölogical Laboratory of the University of Michigan. This is the twenty-second of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

scribed in Part II of this paper, I have proved that the adults of this species develop from *Tetracotyle flabelliformis* Faust, 1917 (March), which in turn developed from *Cercaria douglasi* Cort, 1917 (December). According to the rules of nomenclature, the name of this species, therefore, becomes *Cotylurus flabelliformis* (Faust), a name taken from the first described stage of its life-cycle.

OCCURRENCE AND DISTRIBUTION OF NATURAL INFESTATIONS

In 1925 *C. flabelliformis* was found in domestic ducks on poultry farms both north and south of Grand Rapids, Michigan, and in domestic ducks raised at Ingleside, Michigan. The ducks south of Grand Rapids in which this parasite was found lived in a swampy area that contained only temporary pools. A search of the ground of this area revealed the shells of several species of small snails. Some of them probably served as intermediate hosts of the parasite, but they could become infested with this species only when the swampy ground contained enough water to maintain the aquatic stages of the parasite. This fact suggests the possibility that the eggs of this species may remain viable in moist soil for some time.

A careful comparison of materials showed that probably the same species of *Cotylurus* was present in two wild ducks also, a black duck, *Anas obscura* Gmelin, at Grand Rapids, Michigan, and a lesser scaup duck, *Marila affinis* (Eyton), at Holland, Michigan. During the autumn migrations of 1926 and 1927 a similar parasite was found at Warsaw, Illinois, in a red-legged black duck, *Anas rubripes* Brewst., a pintail duck, *Dafila acuta* (Linn.), a lesser scaup duck, *Marila affinis* (Eyton), five specimens of golden eye, *Clangula clangula americana* (Bonaparte) and two shoveler ducks, *Spatula clypeata* (Linn.). The ducks from Illinois were identified by Mr. Earl Lambert, who kindly sent me their intestines in a fixed and preserved condition. Owing to the fact that the specimens of *Cotylurus* of all the wild ducks had been dead for some time before they were fixed and preserved, I cannot be entirely certain that they are of the same species as the ones obtained from the domestic ducks, although they appear to belong to

it. If this interpretation is correct, the finding of this species in ducks from these localities indicates that it is widespread in this country. From the foregoing records of its distribution it seems probable that *C. flabelliformis* may be found in ducks of any region of North America containing swampy areas, lakes, ponds or streams which are visited by wild ducks during their migrations.

SPECIFIC DIAGNOSIS

Cotylurus flabelliformis (Faust). — Characters of the genus; forebody distinctly set off from the hindbody by a deep groove; forebody nearly spherical, 0.20–0.28 mm. long, *strongly flexed upon the slightly curved, cylindrical hindbody*; the latter, 0.36–0.57 mm. long, broadly truncate at the posterior end; diameter of the forebody, 0.22–0.32 mm., of the hindbody, 0.20–0.26 mm.; one to four lappets of the holdfast organ often protruded from the opening of the forebody; oral sucker terminal, 0.05–0.07 by 0.04–0.08 mm., nearly equal to the acetabulum, 0.06–0.08 by 0.04–0.08 mm.; ovary, flattened-ellipsoidal, mean diameters 0.109 by 0.066 by 0.051 mm., situated in the anterior fourth of the hindbody; the testes, in anterior view bean-shaped deeply incurved at the hilus, in lateral view heart-shaped with the apex directed dorsally; vasa efferentia united into vas deferens in front of anterior testis; eggs, after laying, 0.10–0.112 by 0.068–0.076 mm.; habitat, intestines of domestic and wild ducks of North America.

DESCRIPTION OF THE ADULT

The adult *C. flabelliformis* differs in several respects from the adult *S. tarda* as described by Mathias. The description of *S. tarda* by Mathias is general, however, and lacks many details of taxonomic value, so that a nice comparison is impossible. On this account also no attempt is made here to locate *S. tarda* in the revised classification of Szidat and the name *S. tarda* is retained for Mathias' species. Szidat makes *S. tarda* synonymous with *C. cornutus*, but the discussion presented in this paper will show that such synonymy does not follow from Mathias' description.

The more significant differences between *C. cornutus* and *C. flabelliformis* will also be indicated.

External features. — The body of *C. flabelliformis* is divided by a deep constriction into two body regions, a cylindrical hindbody and a cup-shaped forebody, the former twice as long as the latter. The body is usually strongly flexed at the junction of the body regions, so that the forebody appears to be attached to the antero-dorsal side of the hindbody. Neither Mathias nor Szidat describes such a marked body flexure for their species and their figures do not indicate it. Often the dorsal side of the forebody is extended and its ventral side is contracted so that the opening of the cup appears to be on its ventral surface (Fig. 11). Both these conditions are only apparent, however, since the forebody is attached to the anterior end of the hindbody and the opening of the forebody is in reality at its anterior end. From the opening of the forebody in many specimens one to four lappets of the holdfast organ may protrude. The general color of the body is white, except that along the ventral half of the hindbody there is a brassy-colored area, indicating the location of the brownish vitellaria within. The opening of the bursa copulatrix or genital pore is large and funnel-shaped, and located on the dorsal surface near the posterior end of the hindbody. In some specimens grooves are found near the posterior end of the hindbody. Their presence and extent doubtless depend upon the degree of dilatation of the excretory spaces near them.

Measurements. — Comparative measurements of *C. flabelliformis* and of *S. tarda* (measurements by Mathias, 1925, pp. 25–26) are presented in Table I. The measurements of *C. flabelliformis* were recorded from egg-laying specimens obtained from a duck which was infested daily from July 18 to August 5, 1926. On the latter date the duck was killed and the intestine with its contents was fixed in corrosive-acetic in the usual manner. The specimens appeared to be normal, with no evidence of extraordinary shrinkage. Specimens larger than the ones measured were not collected from any of my feeding experiments. Although the details of Mathias' technique are not given and although he does not explain how his measurements were made, it seems improbable that

TABLE I: MEASUREMENTS OF *C. flabelliformis* IN MM. AS COMPARED WITH THOSE OF *S. tarda* AFTER MATHIAS

Item	Number measured	<i>C. flabelliformis</i>				<i>S. tarda</i>	
		Range	Mean	Smallest specimen	Largest specimen	Type specimen	After Mathias*
Total length †.....	10	0.56-0.85	0.716	0.56	0.85	0.68	0.90-1.98
Forebody.....							
Length †.....	10	0.20-0.28	0.239	0.20	0.28	0.24	0.40-0.60
Dorso-ventral diameter ...	10	0.22-0.32	0.278	0.26	0.32	0.28	0.56-0.72
Hindbody.....							
Length.....	10	0.36-0.57	0.477	0.36	0.57	0.44	
Dorso-ventral diameter § ...	10	0.20-0.26	0.258	0.20	0.26	0.23	
Oral sucker.....							
Length.....	10	0.05-0.07	0.059	0.055	0.06	0.06	0.09-0.109
Dorso-ventral diameter ...	10	0.04-0.08	0.06	0.05	0.07	0.06	0.09-0.109
Pharynx.....							
Length.....	10	0.03-0.045	0.037	0.035	0.038	0.04	
Diameter.....	10	0.02-0.04	0.03	0.03	0.04	0.025	
Acetabulum.....							
Length.....	10	0.06-0.083	0.07	0.06	0.07	0.07	0.112-0.133
Dorso-ventral diameter ...	10	0.04-0.08	0.062	0.055	0.06	0.07	0.112-0.133
Ovary.....							
Transverse diameter 	10	0.075-0.135	0.109	0.075	0.127	0.09	0.11
Dorso-ventral diameter ...	10	0.05-0.075	0.066	0.05	0.07	0.07	0.11
Antero-posterior diameter ..	10	0.04-0.06	0.051	0.04	0.05	0.047	0.058
Uterine egg.....							
Length.....	10	0.08-0.10	0.088	0.08	0.08		
Diameter.....	10	0.04-0.06	0.055	0.05	0.05		
Eggs from feces of duck.....							
Length.....	10	0.10-0.112	0.107				0.097-0.117
Diameter.....	10	0.068-0.076	0.0705				0.062-0.080

* Measurements recorded by Mathias (1925); no statement of method given. † Measured upon median sagittal sections. ‡ Measured along middle line of median sagittal sections. § Measured immediately behind posterior testis. || An approximation obtained by adding the thicknesses of several serial sagittal sections.

differences in methods can account for the wide differences between his measurements and mine. Except in the instances noted in the table, the measurements of *C. flabelliformis* were made upon carefully selected median sagittal sections of the body regions and organs indicated, because this method seemed to be the most accurate. Nearly all the measurements of *C. flabelliformis* fall below those of *S. tarda*, as determined by Mathias, and of *C. cornutus*, as described by Szidat. The eggs of these three species resemble one another quite closely in size, but, since eggs of several species of Strigeidae are known to have a wide range of size within the species, measurements of eggs can have only small significance in determining species. Despite similarities in this character, the wide differences in the other measurements presented in Table I indicate that *C. flabelliformis* is distinct from *S. tarda* and *C. cornutus*.

Holdfast organ and adhesive gland. — In this species the holdfast organ consists of two transverse lips, an anterior and a posterior, both of which are partly cleft into nearly equal right and left parts or lappets (Figs. 10, 12–15). These lappets are contractile and consequently often differ greatly in size in preserved specimens. They may extend outside the cup or they may be folded inside in various ways. The lips of the holdfast organ may also surround varying amounts of host tissue. For these reasons sections of the forebodies of different individuals often present confusingly variable pictures. Immediately beneath the holdfast organ there is a rather compact disk-shaped adhesive gland from which large processes of less compact tissue extend into the tips of the holdfast organ. In *C. flabelliformis* the secretion of the adhesive gland seems to have a corrosive or digestive action, since those parts of the intestinal wall of the host which extend into the cup-like forebody are always denuded of their columnar epithelium. Brandes (1890, pp. 552, 556), Muehling (1896, p. 272), Szidat (1929, p. 635) and La Rue (1927, p. 226) have expressed similar opinions on the corrosive action of this secretion in other species of Strigeidae. The removal of the intestinal epithelium bringing the cuticula of the parasite into direct contact with the vascular tissue of the host doubtless facilitates the absorption of the host's

lymph and blood plasma. Further support for this theory of nutrition is derived from the fact that no blood corpuscles were found in the digestive ceca of any of the specimens examined.

Lateral sucking cups. — In metacercariae the lateral sucking cups are thought to be glandular pockets which have no adhesive function, but in the adult of this species these organs become deep pocket-like cavities which are clearly organs of attachment. These pockets are situated posterior to and at the sides of the pharynx and they extend posterad to the level of the acetabulum. Conspicuous muscle strands extend from the bottoms and sides of the pockets to the dorsal wall of the forebody. In sections pieces of host tissue were often seen filling their cavities. The host tissue, without its epithelium, was often so closely adjusted to the lateral sucking cup of the parasite that it was difficult to distinguish the boundary between parasite and host. The clasping action of the lateral sucking cups, together with the muscular contractions of the forebody as a whole and of the anterior part of the cup-like forebody in particular, serve to attach the parasite to its host.

Following the description of Brandes (1890, p. 559) for *Strigea* and of Muehling (1896, pp. 270, 272) for *Cyathocotyle*, I visualize the action of the forebody of the present species in accordance with its structure as follows. During the contraction of the circular muscles of the forebody some of the liquid in its excretory spaces is pressed into the hindbody, which therefore becomes turgid. Then large muscles extending from the bottom and sides of the cup-like forebody to the organs and dorsal wall of the hindbody contract forcibly and bend the hindbody dorsally against the forebody. This action serves to deepen the cup of the forebody and creates a partial vacuum in it and at the same time makes the forebody narrower, so that the wall of the cup and the lips of the holdfast organ are closely pressed against the interlying host tissue. This intimate application of the parasite to the tissues of the host permits the absorption of the fluids of the host by the parasite.

Digestive system. — The digestive system (Fig. 10) is like that usually found in the Strigeidae. The esophagus is nearly as long as the pharynx. In this respect this species differs from *S. tarda*,

in which the intestinal branches arise immediately behind the pharynx. The digestive ceca extend almost to the posterior end of the animal. In the hindbody they are situated ventral to the reproductive organs. In sections they usually contain a uniformly divided material that stains lightly with eosin and looks like the coagulum of some colloid. It is entirely free from the detritus of the intestinal contents of the host, and seems never to contain blood corpuscles or other cellular elements. Mathias (1925, p. 30) has shown experimentally that *Strigea tarda* ingests liquids and substances in solution, but not solid particles. His findings may also explain the uniformity of the material found in the digestive ceca of the present species. *C. flabelliformis*, therefore, seems to employ two methods of obtaining nourishment: first, by the absorption of the nutritive material from the host through the cuticle of the holdfast organ and the inner surface of the cup-like forebody; and second, by the ingestion through the oral sucker and pharynx of some of the liquid contents of the host's intestine or of the digested epithelium of the intestinal wall itself.

Nerve strands (Fig. 17). — These extend posterad from the pharynx lateral to the digestive ceca of the forebody.

Excretory system. — As in *Crassiphiala bulboglossa* mihi (Van Haitsma, 1925, p. 124), there are four main excretory vessels in the hindbody of *C. flabelliformis*, two dorsal and two ventral. The two dorsal vessels lie dorsal to the reproductive organs. They extend forward from the region of the bursa copulatrix and unite into a single median dorsal vessel near the anterior end of the hindbody. Owing to numerous anastomoses with the subcuticular network of excretory spaces on the dorsal side of the animal, they are less distinct than the two ventral vessels, which have fewer anastomoses with the subcuticular excretory network. In an early developmental stage of the adult the courses of the dorsal vessels were traced to their junctions with the ventral vessels (Figs. 6–8) between the bursa and the posterior testis. In sexually mature adults the region between the bursa and the posterior testis is occupied by excretory spaces traversed by numerous parenchyma strands, so that the dorsal vessels here are not so definitely traceable at this stage of development. The two ventral vessels of the

hindbody are much more definite and distinct than the dorsal vessels, since they anastomose less frequently with the subcuticular excretory spaces. These vessels extend, one on the left, the other on the right, along the descending limb of the uterus. They correspond to the "Uterusgefäße" which Krause (1914, p. 132) described for the hemistomes. In the early developmental stage of the adult (Figs. 3-6) they connect with a very short common excretory vessel which opens terminally at the excretory pore, but in the sexually mature adult the posterior parts of the two ventral vessels could not be traced to the excretory pore. The difficulty here is due to numerous anastomoses with the subcuticular excretory spaces and to the presence of many parenchyma strands which traverse the excretory complex in this region. Near the anterior end of the hindbody the two ventral vessels converge and unite to form a short, narrow transverse vessel with which the median dorsal vessel connects (Fig. 10).

From this transverse vessel a short median vessel extends anterad along the ventral side of the forebody, where it soon becomes lost in a large complex of excretory spaces. A similar but somewhat larger vessel joining the median dorsal vessel of the hindbody extends along the dorsal side of the cup. Posterior to the acetabulum this median dorsal vessel of the forebody sends a branch into the anterior lip of the holdfast organ. Another small branch extends through the adhesive gland dorso-ventrally and joins the median dorsal vessel of the forebody with the median ventral vessel of the same body region. This median dorso-ventral vessel of the adhesive gland is also connected with the median dorsal vessel of the hindbody by means of a short antero-posterior vessel that traverses the center of the adhesive gland. Two branches from the median dorso-ventral vessel of the adhesive gland extend anterad, one into each lip of the holdfast organ. These branches soon divide into secondary branches (Fig. 10) whose courses were not completely traced.

As in other strigeids, there is also in the present species a peripheral network of excretory spaces in both forebody and hindbody (Figs. 3-9, 14-16, 18-20). Along the ventral side of the hindbody they are separated from the ventral vessels by the vitellaria. These

spaces are larger in the lateral regions of the hindbody than along its ventral side. In the posterior part of the dorsal side of the hindbody they may be so large as to make the surface bulge out and thus cause superficial grooves to appear between them (Figs. 11-12). For the sake of clearness these peripheral spaces were not represented in Figure 10.

Except for the median dorso-ventral vessel of the adhesive gland the pattern of the excretory vessels of *C. flabelliformis*, as far as it was traced, agrees in the main with the elaborate results of the study of Thoss (1897, pp. 34-42) on the excretory system of *C. variegatus* (= *Holostomum cucullus* Thoss). According to Mathias (1925, p. 27), the excretory system of *S. tarda* consists of a deeply lobed excretory vesicle from each lobe of which a lateral canal extends forward. In *C. flabelliformis* a definite excretory bladder distinct from the four main canals of the hindbody was not observed. Possibly parts of the subcutaneous excretory spaces (Figs. 19-20) correspond to the lateral canals of *S. tarda*. The three-line description of the excretory system of *S. tarda* by Mathias, unaccompanied by figures, is too brief to be clear and makes impossible a thorough comparison of the excretory systems of these two species. Szidat does not describe the excretory system of *C. cornutus*.

The functions of the excretory system appear to be more comprehensive than its name implies. Since this animal has no distinct circulatory system and since the digestive system seems to be inadequate for the distribution of nourishment to the cells of the body, the distribution of nutriment is probably carried out by the excretory system. The assumption of the circulatory function by the excretory system appears still more probable if nourishment is absorbed through the inner wall of the cup-like forebody, as was suggested above.

Male genital system. — The two testes are located in series and occupy the second and third fourths of the hindbody (Fig. 2). In general they are nearly bean-shaped as seen in anterior view, and their concave surfaces are directed ventrally. They are also somewhat thinner in their median region than laterally. In side view the testes are shaped like hearts with their apices turned

dorsally. Owing to their irregular shapes, measurements of the testes were not made. Their shape differs greatly from that of the testes of *S. tarda*, which are described as flattened disks (Mathias, 1925, p. 26).

The vasa efferentia arise from the midventral surfaces of their respective testes. At first the vasa efferentia are small, inconspicuous ducts, usually free from spermatozoa, but after a short distance they are dilated and made conspicuous by the spermatozoa which they contain. These dilatations are not so large and distinct that they deserve the special designation of "seminal reservoirs." The anterior vas efferens (ve_1) arches forward and toward the right over the surface of the anterior testis and then bending backward unites with the posterior vas efferens (ve_2). The posterior vas efferens also bends forward and toward the right from its point of origin and unites with the anterior vas efferens about midway on the ventral surface of the right half of the anterior testis. From this point of union of the vasa efferentia the vas deferens extends mesad and posterad between the vasa efferentia. On the ventral side of the posterior testis the vas deferens becomes dilated into the large spindle-shaped seminal vesicle. This vesicle practically fills the space formed by the arch of the posterior testis. Posteriorly and dorsally the seminal vesicle empties into the globular thicker-walled ejaculatory pouch. From this pouch the spermatozoa are ejected through a narrow, coiled ejaculatory duct that opens into the genital atrium dorsal to the opening of the uterus. The genital atrium opens to the outside through the large genital pore which is located dorsal to the smaller terminal excretory pore. The ejaculatory duct traverses a mass of clavate, glandular cells like the cells of Mehlis' gland. At first it was thought that these cells represent the prostate gland. However, since their function is not definitely known and since they appear to be similar to the subcuticular gland cells (Krause, 1914, p. 106), it may not be justifiable to characterize them as a prostate gland. Muehling (1896, p. 274) describes such a prostate for *Cyathocotyle prussica* Muehling, and Thoss (1897, p. 49) a similar one for *C. variegatus* (Crepl.). The prostate which Brandes (1890, p. 563) attributes to the genus *Diplostomum* appears to be a mor-

phologically different organ and in fact has not been found by recent investigators.

According to Mathias (1925, p. 26), the vasa efferentia of *S. tarda* arise from the anterior parts of their respective testes. According to my observations, the vasa efferentia of *C. flabelliformis* originate from the midventral points of their testes. In fact, the anterior vas efferens could be traced to a point on the anterior testis near the posterior ventral angle of a median sagittal section. It is regretted that Mathias does not describe where the anterior and posterior vasa efferentia unite, since the location of this point of junction differs greatly according to the descriptions of different authors, e.g. Brandes (1890, p. 562), Thoss (1897, p. 47), Guberlet (1922, pp. 10, 12), La Rue (1926, p. 29) and Augustine and Uribe (1927, p. 239). In *C. flabelliformis* the relations of these ducts closely resemble the arrangement as found in *Neodiplostomum cochleare* (Krause), *Diplostomum huronense* (La Rue), *Pharyngostomum cordatum* (Diesing) according to La Rue, and *Alaria arisaemoides* Augustine and Uribe (references above). The close similarity in the arrangement and connections of these ducts in five genera of the Strigeidae makes the descriptions of Brandes for Strigeidae, of Thoss for *C. variegatus*, and of Guberlet for *D. gavium* and *D. confusum* appear doubtful. Mathias does not describe the presence of an ejaculatory pouch and an ejaculatory duct in *S. tarda*. In *C. flabelliformis* these ejaculatory organs are readily distinguished from the seminal vesicle, since they are thicker-walled.

Szidat (1929, p. 646) describes the course of the male genital ducts as follows. "Nur bei der Gattung *Cotylurus*, soweit ich feststellen konnte, verlaufen die Ausführungsgänge des männlichen Geschlechtsapparates einfacher. Hier zieht das Vas efferens des oberen Hodens sogleich nach unten und nur dasjenige des unteren Hodens hat noch die Richtung nach oben beibehalten (Abb. 13). Die beiden Vasa efferentia vereinigen sich hier etwa in Höhe des Dotterreservoirs." Szidat's Figure 13 of *C. cornutus* entirely confirms his description. It has already been explained why this course of the male genital ducts seems improbable, but if one assumes that Szidat's description of these ducts is correct,

it clearly indicates that *C. flabelliformis* is a distinct species. The different course of the male genital ducts of *C. flabelliformis* also demonstrates that Szidat's conception of the course of these ducts in *C. cornutus* is not universally characteristic of the genus *Cotylurus*. Szidat recognized a muscular ejaculatory duct in *C. cornutus*, but did not distinguish an ejaculatory pouch.

Female genital system (Fig. 1). — The ovary is ovoid and lies on the anterior testis in the median region of the body. The oviduct, arising from the posterior side of the ovary, usually forms a vertical coil before it gives rise to Laurer's canal. However, when the hindbody straightens this coil disappears. Laurer's canal extends along the middorsal line to the region between the two testes where it opens to the outside. The oviduct accompanies Laurer's canal along its ventral side, but in the region between the two testes it turns sharply ventrad into Mehlis' gland. Here the oviduct is joined by the common vitelline duct and then becomes the oötype which extends through Mehlis' gland from right to left. From Mehlis' gland the ascending limb of the uterus continues ventrad and anterad between the posterior vas efferens and the vas deferens along the right side of the ovary to the anterior end of the hindbody. Bending abruptly ventrad and posterad it continues, as the descending limb of the uterus, in the median plane to the posterior end of the hindbody. It extends throughout its length between the two ventral excretory vessels and in the region of the posterior testis lies ventral to the vas deferens. It opens into the genital atrium immediately ventral to the opening of the ejaculatory duct.

Mehlis' gland lies between the testes. In *C. flabelliformis* it is less compact than in some other species of Strigeidae and consequently the fibrous network which binds its single-celled glands together is more readily observed in this species. In *C. flabelliformis* it consists of a large irregular mass of glandular cells. Mathias did not see Mehlis' gland in *S. tarda*.

The vitellaria are found in a single field along the ventral half of the hindbody between the intestinal ceca and the subcuticular excretory spaces. The rather large follicles are connected with the vitelline reservoir by means of right and left vitelline ducts,

which cross the inner sides of the digestive ceca. Unless they contain vitelline material these small ducts are easily overlooked. The vitelline reservoir located on the left of Mehlis' gland is nearly triangular both in anterior and sinistral views. Near its dorsal angle the vitelline reservoir gives rise to the common vitelline duct, which extends dorsad and dextrad around the oviduct and then bending ventrad and sinistrad empties into the oviduct.

Genital atrium. — This is the cavity of the bursa copulatrix. It is irregular in shape, principally owing to a broad, tongue-shaped muscular process extending inward from its anterior wall ventral to the opening of the uterus. This muscular process is Szidat's chief distinguishing characteristic of the genus *Cotylurus*. This process ordinarily nearly fills the genital atrium in *C. flabelliformis*. Its function is not definitely known, but it appears to be of a special nature, since it is not present in all species of Strigeidae (Krause, 1914, p. 190, and Van Haitsma, 1925, p. 126). Its structure and position suggest that it may function during the extrusion of the egg or during copulation. Mathias does not mention it in his description of *S. tarda*. According to the only record of observed copulation in the Strigeidae (Brandes, 1890, p. 566), the bursa copulatrix serves as a copulatory organ.

SYNOPSIS OF DIFFERENCES BETWEEN *C. flabelliformis*
AND MATHIAS' DESCRIPTION OF *S. tarda*

1. Measurements of *C. flabelliformis*, the whole organism, the body regions and various organs, fall below the limits of corresponding measurements of *S. tarda*.

2. In *C. flabelliformis* the esophagus is nearly as long as the pharynx (Fig. 17). In *S. tarda* the intestinal branches arise immediately behind the pharynx.

3. There are four main excretory vessels in the hindbody of *C. flabelliformis*, two dorsal and two ventral. Near the posterior end of the hindbody these vessels anastomose without forming a distinct excretory bladder. The excretory system of *S. tarda* consists of a deeply lobed excretory bladder, from each lobe of which a lateral excretory canal runs forward.

4. The testes of *C. flabelliformis* are bean-shaped organs, thinner medially than laterally. The testes of *S. tarda* are flattened disks.

5. A Mehlis gland appears to be lacking in *S. tarda*. In *C. flabelliformis* an irregularly shaped Mehlis gland is found in the usual position.

6. A muscular tongue-shaped process extends into the genital atrium of *C. flabelliformis*. Mathias does not mention such a process for *S. tarda*.

PART II. LIFE-HISTORY

DURING the present decade several life-history studies in the family Strigeidae have been made in South America (Lutz, 1920) and Europe (Ruszkowski, 1921; Mathias, 1922; Szidat, 1923 and 1924), but such a study has thus far not been made in North America. While the present study of the life-history of *Cotylurus flabelliformis* (Faust) was in progress, the dissertation of Mathias (1925, pp. 1-65) on the "Cycle évolutif d'un trematode holostomide, *Strigea tarda* (Steenstrup)" became available. This work of Mathias offered the opportunity to make comparisons in the life-histories of two structurally similar species of different continents, *S. tarda* of Europe and *C. flabelliformis* of North America. A comparative morphological description of these two species has already been made in Part I of this paper.

Snail hosts. — Since *C. flabelliformis* resembles *S. tarda* in form and structure and was found in similar definitive hosts, it was presumed that the life-histories of these two species would not differ greatly. Therefore various snails of Douglas Lake were examined for tetracotyles resembling those described as *Tetracotyle typica* Diesing, the metacercaria of *S. tarda* (Lühe, 1909, p. 163; Szidat, 1923, p. 310). Such tetracotyles were found in *Lymnea humilis* Say, *L. reflexa* Say, *L. stagnalis appressa* Say, *L. stagnalis perampla* Walker, *L. emarginata angulata* Sowerby and *Planorbis trivolvis* Say. Hughes (1929, pp. 495-508) described these tetracotyles from the same species of snails of the same lake as *Tetracotyle flabelliformis* Faust.

TABLE II
FEEDING EXPERIMENTS

Record number of experiment	Record number of duck	Tetracotyles or snails fed	Period of feeding	Death of host	Results
Group A					
I	111	About 100 <i>P. trivolvris</i>	July 20 July 25, 1925	Died July 25, 1925	100 + Cotylurus, mature and immature
II	113	About 100 <i>L. emarginata angulata</i>	July 18-July 25, 1925	Died July 25, 1925	100 + Cotylurus, mature and immature
III	130	About 200 <i>L. stagnalis appressa</i>	July 18-Aug. 5, 1925	Killed Aug. 5, 1925	100 + Cotylurus, mature and immature
IV	171	* <i>L. emarginata angulata</i>	July 10, 1926	Killed July 16, 1926	No Cotylurus, no fecal examination
	202	* <i>L. emarginata angulata</i>	July 10, 1926	Killed Aug. 10 1926	No Cotylurus, no fecal examination
	217	* <i>L. emarginata angulata</i>	July 10, 1926	Killed Oct. 7, 1926	No Cotylurus, no fecal examination
V	186	† <i>L. stagnalis appressa</i> and <i>P. trivolvris</i>	July 1 Aug. 2, 1926	Killed Aug. 2, 1926	100 + Cotylurus, mature and immature
	212	† <i>L. stagnalis appressa</i> and <i>P. trivolvris</i>	July 1-Aug. 7, 1926	Killed Aug. 28, 1926	No Cotylurus, but collected eggs
	214	† <i>L. stagnalis appressa</i> and <i>P. trivolvris</i>	July 1-Aug. 7, 1926	Died Sept. 7, 1926	No Cotylurus, but collected eggs
VI	248	60 <i>L. stagnalis appressa</i>	July 28-Aug. 3, 1927	Killed Aug. 4, 1927	25 + Cotylurus, mature and immature

TABLE II (continued)

Group B VII	232	20 tetracotyles without cyst-like coverings from <i>L. stagnalis appressa</i>	July 11, 1927	Died July 14, 1927	No <i>Cotylurus</i> , no fecal examination
VIII	254	30 tetracotyles without cyst-like coverings from <i>L. stagnalis appressa</i>	Aug. 4, 1927	Killed Aug. 8, 1927	No <i>Cotylurus</i> , no fecal examination
IX	246	235 tetracotyles with cyst-like coverings from <i>L. reflexa</i>	July 2-July 23, 1927	Died Aug. 3, 1927	No <i>Cotylurus</i> , but eggs were collected on July 9
X	269	40 tetracotyles with cyst-like coverings from <i>L. stagnalis appressa</i>	Aug. 9, 1927	Killed Aug. 15, 1927	No <i>Cotylurus</i> , no fecal examination
Group C XI	270	13 <i>L. stagnalis appressa</i> , all tetracotyles have cyst-like covering	Sept. 6, 7, 1927	Killed Sept. 10, 1927	200 + <i>Cotylurus</i> , mature and immature, also eggs collected on Sept. 10

* Five snails to three ducks.

† Five hundred and twenty-nine snails to three ducks.

General technique of experiments. — During the summers of 1925, 1926 and 1927 the four species of snails last mentioned were selected for eleven feeding experiments involving fifteen domestic ducks, as recorded in Table II. During the course of the experiments the ducks, from three to seven weeks old, were carefully guarded against other than purposive strigeid infestations. They were kept on sand in pens screened with poultry netting, about four hundred feet from the lake shore, where it was impossible for them to obtain any materials which might contain strigeid metacercariae except those fed. No attempt was made to screen the ducks against insects, since there are no records of the finding of metacercariae of the Strigeidae in insects. The food of the ducks consisted of bread, lettuce, table scraps, prepared chick mash, and bread and milk mixed with sand. Occasionally a few scraps of fresh beef were also fed. The drinking water was pumped from a well. The snails fed to the ducks in the experiments were always crushed just before feeding. When tetracotyles alone were to be given to the ducks they were picked from the crushed snails, examined and sorted microscopically and introduced into the ducks either by means of a pipette or by placing them in small cavities formed in pieces of beef, which the birds ate greedily. The feces of the ducks were examined for strigeid eggs at the beginning of each experiment to determine whether the birds were free from parasites. Fecal examinations were also made at intervals during some of the experiments either for the purpose of collecting the eggs or to watch the progress of an infestation. At the end of each experiment and at different intervals after the last experimental feeding the ducks were killed and their intestines were examined for their trematode populations. Thereupon the intestines with their contents were shaken in water for three minutes, then in corrosive-acetic for one-half minute, and five or more hours later any trematodes that were present were sorted out of the intestinal contents, washed in water, and preserved in 70 per cent alcohol with glycerine.

Grouping and general description of experiments. — According to the materials fed my infestation experiments fall into three groups, A, B and C. In group A, experiments I–VI, naturally

infested snails from Douglas Lake were fed to ten ducks. The experiments of group B consist of two subgroups, B' and B''. In subgroup B', experiments VII and VIII, tetracotyles without cyst-like coverings were removed from the snails and fed to two ducks; in subgroup B'', experiments IX and X, encysted tetracotyles were similarly removed from snails and fed to two ducks. In group C, experiment XI, snails experimentally infested with *Cercaria douglasi* Cort, 1917, were fed to one duck. The experiments of group A were made during the summers of 1925, 1926 and 1927. They prove that tetracotyles naturally infesting snails of Douglas Lake develop into adult *C. flabelliformis* in the domestic duck. The experiments of group B showed that encysted tetracotyles of this species continued their development in the domestic duck while the tetracotyles without cyst-like coverings did not develop. The experiment of group C demonstrated not only that *Tetracotyle flabelliformis* Faust, 1917 (March), continued its development in the domestic duck, but also that this tetracotyle itself developed in the snails from *Cercaria douglasi* Cort, 1917 (December). In order to evaluate the significance of these groups of experiments it will be necessary to discuss them in somewhat greater detail.

Group A

Experiments I, II and III. — The ducks of these three experiments were infested with trematodes when they came into my possession. This was determined by fecal examinations before the feedings began. In the feces of each of the three ducks a small number of strigeid eggs were found. The history of these ducks during the three days previous to the fecal examinations was known to have been such that infestation during that time was impossible. During two of the three days the ducks were in transit and on the third day they were in the pens and cared for as related in the general description of technique. My later investigations have shown that *C. flabelliformis* attains the egg-laying stage in about three days after entering the duck. Hence, if the trematodes present in these ducks were *C. flabelliformis*, they all must have attained the egg-laying stage before the be-

ginning of the experiments. Since the fecal examinations revealed only a few eggs, it is probable that the number of strigeids present in the ducks at this time was small. During these three experiments, I, II and III, snails containing tetracotyles were fed daily to the ducks until the time of their death. On the fourth day after the first feeding with the snails the number of eggs in the feces of the ducks had increased very greatly. There is no doubt that this increase was due to a greater number of adult worms secured from the snails fed. Moreover, in each of these experiments specimens of *C. flabelliformis* were finally obtained in all stages of development from slightly modified tetracotyles to the egg-laying adults. The finding of this graduated series of stages in all three experiments is conclusive proof that increased infestation resulted from the experimental feeding.

Experiment IV. — This experiment of group A was not successful. Five snails containing tetracotyles were fed to three ducks on one day. Circumstances prevented the carrying out of this experiment as originally planned. No fecal examinations were made and the ducks were killed and examined more than a month after the experimental feeding. At this time these ducks were parasite-free.

Experiment V. — Since the three ducks of this experiment were under my care from day-old ducklings until their death, this is a critical experiment. One of the ducks was fed snails containing tetracotyles from the time that it was three weeks old until it was killed five weeks later. Examination of its intestines revealed that it contained more than one hundred specimens of mature and immature *C. flabelliformis*. The record of this duck, therefore, shows without question that the tetracotyles in the snails developed into adults in the domestic duck. Fecal examinations during the course of the feeding of the other two ducks showed that they also became infested. Owing to the belief that the adults obtained from experiments I, II and III of group A were not full-grown, the two remaining ducks of this experiment were kept about one month after the last feeding of snails to permit their parasites to grow to a larger size, but at the time of their death they had become parasite-free.

Experiment VI. — The duck of the last experiment of group A was six to seven weeks old at the time when the experimental feeding began. During the last two weeks previous to this time it had been under my care. The conditions under which it lived before it came under my care are not known. Absence of eggs from the feces at the beginning of the experiment showed that it was parasite-free. By actual count sixty *Lymnea stagnalis appressa* were fed to it during the six days prior to its killing. The post-mortem examination revealed twenty-five mature and immature *C. flabelliformis* in its intestines. The result of this experiment agrees, therefore, with the results of experiments I, II, III and V.

Conclusions from the experiments of group A. — The preceding experiments, constituting group A, show that tetracotyles in *L. stagnalis appressa*, *L. stagnalis perampla*, *L. emarginata angulata* and *Planorbis trivolvis* are able to develop to maturity in the domestic duck. These tetracotyles, although found in four kinds of snails, belong to one species because they develop into one kind of adult. Since they were found in the snails early in the summer as soon as the snails came up from deep water, it seems probable that this species passes the winter in the snail in the tetracotyle stage. The number of adult parasites recovered from the ducks for each snail fed is small. The duck of experiment VI was older than the ducks of the other experiments and the ratio of adults recovered from it as compared with the number of tetracotyles fed was smaller than in the experiments with the younger ducks. This result agrees with the belief already held that older ducks are less readily infested than younger ones.

Group B

General considerations. — In the snails of the experiments of group A two kinds of tetracotyles were found, some with thick, gelatinous, cyst-like coverings and others without such coverings, but only one kind of adult was obtained from the ducks to which both kinds of tetracotyles were fed. Therefore the experiments of group B (VII, VIII, IX and X) were devised to determine which of these kinds of tetracotyles, those with or those without the

thick coverings, had developed in the ducks of group A. They were removed from the snails and sorted as already described. The collection of a dose of tetracotyles required from one to two hours, during which time they were kept in water in watch glasses. The doses were given to the ducks imbedded in small pieces of beef, or in water by means of a pipette. The ducks of these experiments were under my care more than a week before the experimental feedings began. Fecal examinations during this time showed that all of them were free from parasites.

Experiments VII and VIII. — Small doses of tetracotyles without cyst-like coverings were given to the ducks of experiments VII and VIII and a few days later the ducks were examined *post mortem* for the *Cotylurus* they might contain. No parasites were found in either duck. The most probable explanation of this result is that tetracotyles without the cyst-like coverings were too immature to continue their growth in the domestic duck. Conclusive evidence will be presented in connection with the discussion of experiment XI of group C to show that tetracotyles without cyst-like coverings develop into tetracotyles with cyst-like coverings. *A priori* it seems probable that the thick gelatinous covering is essential for the protection of the delicate tetracotyle against the grinding and digestive power of the gizzard of the duck. The interpretation that tetracotyles without cyst-like coverings are too immature to develop in the duck is supported by von Linstow (1894, p. 332), who described the formation of a similar cyst for *Tetracotyle typica* and expressed the belief that *Tetracotyle typica* can become sexually mature in vertebrates only after the cyst is formed. There is, therefore, sufficient reason to believe that tetracotyles without cyst-like coverings cannot develop in the domestic duck, yet it must also be admitted that experiments VII and VIII, owing to the small number of tetracotyles fed, were not sufficiently critical to establish this belief beyond all doubt.

Experiments IX and X. — In each of these two experiments tetracotyles with cyst-like coverings were fed to a duck. To duck 246 of experiment IX two hundred and thirty-five were fed during a period of three weeks, July 2 to July 23. On July 9, just one week after the feeding began, strigeid eggs were observed in its

feces, proving that tetracotyles with cyst-like coverings had developed into adults, yet at the autopsy of the duck on August 3, eleven days after the last experimental feeding, no parasites were found in its intestine. Presumably the period of eleven days was longer than the life of the parasites in the duck. In experiment X only forty tetracotyles with cyst-like coverings were fed to a duck on one day, August 9, and six days later, on August 15, the duck was killed. At the post-mortem examination no parasites were found. Their absence needs to be explained.

The explanation for the negative result of experiment X is suggested by my previous experiments, namely, that too few tetracotyles were used in feeding. All my feeding experiments show that only a small percentage of the tetracotyles ingested by the ducks underwent development. In the experiments giving positive results the number of tetracotyles fed was always many times greater than the number of adults obtained and in experiments involving small numbers of tetracotyles infestation did not take place at all. Although the number of tetracotyles in each of the snails fed is not known, an actual count of the number found in thirteen snails by Hughes (1929, p. 495) gave an average of one hundred and eighteen per snail. Hence an average of fifty fully developed tetracotyles in each snail is a conservative estimate. On the basis of this estimate less than two per cent of the tetracotyles fed develop to maturity in the domestic duck. For this reason experiments IV, VII, VIII and X do not give conclusive results. At most, there is only a slight indication in experiments VII and VIII that tetracotyles without cyst-like coverings do not develop in the domestic duck. That tetracotyles with cyst-like coverings do develop in ducks is clearly demonstrated by my experiment IX and also, as will be shown later, by experiment XI.

Group C

Life-history experiment. Experiment XI. — The development of *Cercaria douglasi* Cort into adult *C. flabelliformis* has been demonstrated thus. During the summer of 1927 Professor W. W. Cort permitted me to utilize the results of his experiments on the

development of *Cercaria douglasi* in snails. A brief note concerning these experiments has been published (Cort and Brooks, 1928, p. 194). The details are as follows: A lot of snails, *L. stagnalis appressa* Say and *L. stagnalis perampla* Walker, were collected from a limited area of the north shore of Douglas Lake. Twenty-five, used as controls, were examined for tetracotyles, but none of them showed an infestation. From July 26 until July 29 forty were exposed to large numbers of cercariae. At intervals Professor Cort killed and examined a few and in this way was able to watch the development of the penetrated cercariae into the mobile metacercaria stage, i.e. the stage before the thick gelatinous covering was formed. On August 17 examination of three snails indicated that all the cercariae had attained this stage of development. All of Professor Cort's observations showed that the metacercariae resulted only from his experimental infestations.

On August 19, 1927, Professor Cort generously turned over to me twenty-eight of these snails which had been experimentally infested about three weeks before. They were carefully tended until September 6, but in spite of excellent care fourteen died during this time. On the 6th and 7th of September, about six weeks after their exposure to *Cercaria douglasi*, thirteen of the fourteen remaining snails were examined. After removal of the shell the posterior twisted part of the body of each snail was teased apart and the tetracotyles present were studied under the dissecting microscope. The number in each snail was extraordinarily large, much larger than the number usually found in naturally infested snails of Douglas Lake. Apparently the tetracotyles were identical with those employed in my former experiments. Every one observed possessed a thick gelatinous covering. After the examination of each snail it was immediately fed, together with its tetracotyles, to a five-week-old duck (number 270 of experiment XI in Table II), which had been in my possession for only one day before this feeding experiment began. On both September 6 and 7 the feces of this duck were examined for strigeid eggs, but none were found. On September 10, however, more than two hundred strigeid eggs were collected from the feces. On the evening of September 10 the duck was killed and more than two

hundred mature *Cotylurus* were obtained from its intestine. Careful study showed that these parasites agreed in all respects with those obtained as a result of my other feeding experiments in which I used naturally infested snails. Therefore, I conclude that all the strigeid parasites obtained from my experiments must have developed from *Cercaria douglasi* Cort, 1917.

The life-cycle. — Cort and Brooks (1928, p. 191) obtained *Cercaria douglasi* from *Physa sayii oneida* Baker, *Physa parkeri* "Currier" De Camp, *Lymnea emarginata angulata* Sowerby, *L. stagnalis appressa* Say and *L. stagnalis perampla* Walker. This list, therefore, also indicates certain snails into which the miracidia of this species penetrate. Moreover, I have collected the eggs of this species and observed the development of the miracidia within the eggs. Hence all the salient stages of the life-history of *C. flabelliformis* have been seen and the hosts of a complete life-history are known. My observations on this species and on the life-cycle of another species of *Cotylurus* which has been experimentally determined (Van Haitsma, 1930), together with the data of Mathias (1925, p. 63) on *S. tarda*, permit me to outline briefly the life-cycle of *C. flabelliformis*. In a general way this outline will probably apply to all species of Strigeidae except that in most species the second intermediate host is a vertebrate. The periods of time suggested in this outline imply summer temperatures.

<i>Stage of Development</i>	<i>Time Required</i>
Egg develops into miracidium.....	about 3 weeks
Miracidium swims in the water and penetrates into first snail host.....	a few hours
Miracidium metamorphoses into mother sporocyst, which gives rise to secondary sporocysts that produce cercariae.....	about 6 weeks (Mathias)
Cercariae escape into water and penetrate into second snail host.....	within 1 day
Cercariae develop into tetracotyles within second snail host.....	about 6 weeks
Second snail host ingested by a duck in which tetracotyles develop into egg-laying adults.....	3 or 4 days
Egg-laying stage within the duck.....	about 1 week

It is possible, therefore, under favorable conditions for the whole life-cycle to be completed within four months, but during the winter the process of development doubtless continues at a much slower rate and may even come to a standstill. Eggs which were kept at low temperatures for three weeks did not develop noticeably, but when the temperature was raised to 23° C. development again proceeded. Cercariae also become less active as the temperature decreases, yet the temperature requirement for the penetration of strigeid cercariae into the second intermediate host is considerably less than that of schistosome cercariae. There is good reason to believe that the tetracotyles pass the winter within the snail host, since they may be found in large numbers in the snails in early summer. As already explained, the stage within the final host attains sexual maturity very soon and lives only a short time. The periods suggested for the adult in the outline above are doubtless too short for many other Strigeidae.

The life-cycle of *C. flabelliformis*, as far as studied, is closely similar to that of *S. tarda*. The stages within the first snail host were not actually studied by me, but doubtless these two species are also similar with respect to this part of the life-cycle. Hence the description of these stages in the outline above was confidently paraphrased from Mathias' description of *S. tarda*. In a species of *Diplostomum*, syn. *Proalaria*, and another species of *Cotylurus*, the period from the penetration of the miracidia to the appearance of the cercariae was also about six weeks.

The determination of this life-cycle corroborates the fact developed since 1920 (La Rue, 1926, pp. 269-271) that the Strigeidae are not metastatic, as was supposed from 1889 until 1920. In the strigeid life-cycle there is an alternation of generations as well as an alternation of hosts. While the number of eggs produced by the hermaphroditic adult is comparatively small, the multiplication within the first snail host is enormous. Under favorable conditions a few infested ducks remaining on a lake for a few days may during the period of the life-cycle of the present species infest the snails of the lake with millions of tetracotyles. In this way this species and others of the family Strigeidae are well

adapted for distribution by migratory birds, which may remain on one body of water for only a short time.

Experiments with chicks. — During the summer (1928) following that in which the life-history of this species was determined, attempts were made to develop tetracotyles in chicks. Two chicks, only two weeks old at the beginning of the experiment, were fed from twenty to fifty tetracotyles a day from June 29 to July 7. The tetracotyles taken from the snails were placed on moistened bread and fed in this way. Fecal examinations, both before and after this experiment, showed that no eggs were present. On July 7 one of the chicks was killed and examined. Its intestines were free from parasites. Experimental feeding of the other chick was discontinued till it was about seven weeks old. Beginning on August 3 crushed snails, thirty-six in all, were fed to this chick during three days. On August 7 a fecal examination revealed the presence of strigeid eggs. On August 8 the chick was killed and examined. Its intestines contained about fifty mature and nearly mature *C. flabelliformis* similar in all respects to those obtained from my experimental ducks. This experiment demonstrates, therefore, that in chicks of the proper age the parasite develops to maturity just as readily as in domestic ducks.

TRANSIT DEVELOPMENT

Three characteristics of *C. flabelliformis* deserve special attention, since they have a bearing upon the interpretation of its life-cycle. These are of small size, early sexual maturity and short life. Mature specimens are smaller than those of all other known species of the former genus *Strigea*, being noticeably smaller than *S. tarda* as described by Lühe (1909, p. 163) and Mathias (1925, p. 25). Associated with its small size is an early sexual maturity. In my experiments I, II, III and XI, and in the experiment with the chick, numerous eggs were found in the feces on the fourth day after the first experimental feeding. Mathias (1925, p. 24) found some eggs of *S. tarda* in the feces of his experimental ducks on the third day after infestation, but Lutz (1920, pp. 128-129)

states that the species which he hastily supposed to be *S. tarda* required about ten days after infestation to attain the egg-laying stage. The small size and early sexual maturity of *C. flabelliformis* are probably physiologically correlated with its short life. In several of my experiments this species remained in the ducks a much shorter time than the monostomes and echinostomes which were also present. Lutz (1920, p. 128) predicted that some of the smaller holostomes (Strigeidae) would prove to remain in their definitive hosts only a short time. Mathias (1925, p. 64) found that a mass infestation of *S. tarda* in domestic ducks did not last more than twelve to eighteen days. In my experiment VII the parasites left the duck within eleven days after the last experimental feeding. Now all three of these characteristics of the adult *C. flabelliformis*, small size, early sexual maturity and short life, are also characteristic of cases of transit development in the sense that this term was employed by Faust and Nishigori (1926, p. 94). It is pertinent, therefore, to inquire whether the development of *C. flabelliformis* in the domestic duck represents a case of transit development.

Before attempting to answer this question, it is desirable to consider the percentage of tetracotyles which develop into adults in domestic ducks and also the lack of host specificity of *C. flabelliformis* and *S. tarda*. According to a previously discussed estimate, less than two per cent of the tetracotyles fed to the domestic duck succeed in developing to the egg-laying stage. Although the percentage of viability is not known and definite records of the percentage of tetracotyles of other species of strigeids which develop to the egg-laying stage in their definitive hosts are not available, yet my experience in more than twenty successful feeding experiments involving five species of holostomes leads me to believe that the percentage of tetracotyles of the present species which develop into egg-laying adults in the domestic duck is extraordinarily small. Other conditions being equal, a low percentage of development of tetracotyles in the definitive host indicates a lack of adaptation between parasite and host. Such a lack of adaptation is favorable to the interpretation of transit development.

There are also several phenomena indicating a lack of host specificity of *C. flabelliformis* and *S. tarda* which are significant of transit development. Cort and Brooks (1928, p. 191), after naming the different kinds of snails from which they obtained the cercaria of *C. flabelliformis*, declare that "the lack of specificity displayed by this species in its development in so many different hosts we believe goes beyond anything yet reported for holostome cercariae." To this declaration may be added that the lack of specificity of the cercaria is matched by that of the tetracotyle, which also has been found in five species of snails belonging to two genera. Doubtless the list of hosts of the cercariae and metacercariae of this species is not yet complete. So far, the adult *C. flabelliformis* has been found in seven species of ducks belonging to five genera and it has been experimentally developed in a chicken, but if its similarity to *S. tarda* includes also a similar diversity of definitive hosts, we may expect to find it in a much greater variety of hosts. By experimental feeding Mathias (1925, p. 32) obtained a complete development of *S. tarda* in both domestic and wild ducks and also in a quail. Hence it seems probable that the present species, which closely resembles *S. tarda* in its life-history, may be found in more kinds of hosts. If among these hosts there should be found one in which a greater percentage of tetracotyles would develop to sexual maturity, grow more slowly and to a larger size, and live longer, there would be sufficient evidence to conclude that the development of *C. flabelliformis* in ducks is a case of transit development, but as long as such evidence is lacking it can only be said that the development of this species in domestic ducks resembles cases of transit development.

Pathology in the snail. — The life-history experiments described above demonstrate clearly that snails heavily infested with the cercariae of *C. flabelliformis* may be killed by the developing tetracotyles. Of the snails experimentally infested by Professor Cort, fifty per cent died before the tetracotyles were fully developed, while other snails, not experimentally infested and kept under similar or even less favorable conditions, showed a comparatively low death-rate. Faust (1920, pp. 1-16) has made

a careful study of pathological conditions resulting from the development of sporocysts and cercariae in snails. If similar pathological conditions are produced by developing tetracotyles, they would readily account for the high death-rate of heavily infested snails.

Pathological symptoms in the ducks. — Several symptoms of disease were observed in my experimental ducks and during the course of the experiments four of the nine heavily infested ones died. These symptoms may be summarized as follows: (a) paresis, indicated by a leg weakness and extraordinary tendency to sit; (b) muscular incoördination, resulting in inability to direct locomotion; (c) nervous twitchings of the head and wings; (d) dyspnea, indicated by a labored expansion and contraction of the body; (e) diarrhea and (f) irregular appetite. Two apparently healthy ducks which were given extraordinarily heavy feedings of snails containing tetracotyles suddenly died within a week without showing definite symptoms. My observations of symptoms are confirmed by similar observations of Katsurada (1914, p. 310), Galli-Vallerio (1914, pp. 97–115) and Tabangui (1926, p. 5) in helminth infestations.

Although it seems probable, therefore, that at least some of the symptoms of disease in my young ducks were due to the parasitic infestations, it is also not improbable that some of the symptoms observed were due to cold and dampness or to improper feeding. Equally severe infestations did not always produce the same symptoms in ducks of the same age. Besides, control experiments for the definite purpose of relating the symptoms of disease to the parasites were not performed. Moreover, Dr. Ernest Hartman discovered a species of *Leucocytozoön* in some of my ducks. This double infestation makes the problem of explaining the symptoms twice as complicated, especially so since the pathological effects of *Leucocytozoön* on the domestic duck is not known (Wenyon, 1926, p. 908).

Although it is impossible at present to attribute definitely any symptom of disease in my ducks to *C. flabelliformis*, my observations suggest the opinion that well-directed and controlled experiments on large numbers of ducks will demonstrate that there is

a connection between some of the symptoms observed and the parasitic infestations. Mathias held the opposite opinion, but probably he did not observe very heavy infestations. As has already been described (Part I, above), *C. flabelliformis* digests away the columnar epithelium of the intestinal mucosa of its host and causes a congestion of blood in the subepithelial connective tissue of the host's mucous membrane at the points of attachment of the parasites. Consequently, it seems reasonable to believe that large numbers of the parasites should have injurious effects upon their hosts. It also seems plausible that the irritations of the intestinal wall of the duck by the parasites, or possibly by their toxic secretions, should cause some of the nervous symptoms observed.

Summary. — The life-cycle of *C. flabelliformis*, here described, is the first strigeid life-cycle to be determined in North America. The cercaria has previously been described by Cort and Brooks (1928) and the metacercaria by Hughes (1929). The adult is described in Part I of the present paper. It is noteworthy that these stages of development were all secured from the same kinds of snails, from the same lake, during the same summer. The peculiarities of this life-cycle which suggested that it may be a case of transit development are discussed, and the pathological effects that this parasite has or may have upon its hosts are considered.

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LITERATURE CITED

- AUGUSTINE, D. L., AND URIBE, C. 1927. *Alaria arisaemoides* n. sp., a Trematode from *Vulpes fulva*. Parasitol., 19: 236-244, 3 pls., 4 text figs.
- BRANDES, G. 1890. Die Familie der Holostomiden. Zool. Jahrb., Jena Abt. f. Syst., 5: 549-604, 3 pls.
- CORT, W. W. 1917. Homologies of the Excretory System of the Forked-tailed Cercariae. Journ. Parasitol., 4: 49-57, 2 text figs.
- AND BROOKS, S. T. 1928. Studies on the Holostome Cercariae from Douglas Lake, Michigan. Trans. Am. Microsc. Soc., 47: 179-221, pls. 24-28, 2 text figs.
- FAUST, E. C. 1917. Notes on the Cercariae of the Bitter Root Valley, Montana. Journ. Parasitol., 3: 105-123, 1 pl.
- 1920. Pathological Changes in the Gasteropod Liver Produced by Fluke Infection. Johns Hopkins Hosp. Bull., 31: 1-16, 2 pls.
- AND NISHIGORI, M. 1926. The Life-Cycle of Two New Species of Heterophyidae, Parasitic in Mammals and Birds. Journ. Parasitol., 13: 91-128, 4 pls., 4 text figs.
- GALLI-VALLERIO, R. 1914. L'État actuel de nos connaissances sur le rôle pathogène des helminthes. Centralbl. Bakt. . . , 1 Abt., Ref., 61: 97-115.
- GUBERLET, J. E. 1922. Three New Species of Holostomidae. Journ. Parasitol., 9: 6-14, 2 pls.
- HUGHES, R. C. 1929. Studies on the Trematode Family Strigeidae (Holostomidae). No. XVII. *Tetracotyle flabelliformis* Faust. Pap. Mich. Acad. Sci. Arts and Letters, 10: 495-508, 1 pl.
- KATSURADA, F. 1914. Studien über Trematodenlarven bei Süßwasserfischen, mit besonderer Berücksichtigung der Elb- und Alsterfische. Centralbl. f. Bakt. . . , 1 Abt., Orig., 73: 304-314, 1 pl., 10 text figs.
- KRAUSE, R. 1914. Beitrag zur Kenntnis der Hemistominae. Zeitschr. f. wissensch. Zool., 112: 93-238, 1 pl., 78 text figs.
- LA RUE, G. R. 1926. Studies on the Trematode Family Strigeidae (Holostomidae). No. I. *Pharyngostomum cordatum* (Diesing) Ciurea. Trans. Am. Microsc. Soc., 45: 1-10, 2 pls.
- 1926 b. Studies on the Trematode Family Strigeidae (Holostomidae). No. III. Relationships. *Ibid.*, 45: 265-281, 1 pl.
- 1927. Studies on the Trematode Family Strigeidae (Holostomidae). No. V. *Proalaria huronensis* sp. nov. *Ibid.*, 46: 26-35, 2 pls.
- 1927. A New Species of Strigea from the Herring Gull, *Larus argentatus* (Pont.) with Remarks on the Function of the Holdfast Organ. Journ. Parasitol., 13: 226.

- LÜHE, M. 1909. Parasitische Plattwürmer: Trematoden. Brauer's Süßwasserfauna Deutschlands, 17: 1-217, 188 text figs. Jena.
- LUTZ, A. 1920. Zur Kenntnis des Entwicklungszyklus der Holostomiden. Centralbl. f. Bakt. . . ., 1 Abt., Orig., 86 (Heft 2): 124-129.
- MATHIAS, P. 1922. Cycle évolutif d'un trematode holostomide (*Strigea tarda* Steenstrup). C. R. Ac. Sciences, Paris, pp. 599-602.
- 1925. Recherches expérimentales sur le cycle évolutif de quelques trematodes. Bull. Biol. France et Belgique, 59: 1-123, 4 pls.
- MUEHLING, P. 1896. Beiträge zur Kenntnis der Trematoden. Archiv. f. Naturgesch., 62: 242-279, 4 pls.
- RETTGER, L. J. 1896. Some Additions to Our Knowledge of the Anatomy and Embryology of the Holostomidae. Proc. Ind. Acad. Sci., 1896: 224-226.
- RUSZKOWSKI, J. 1921. Postembrjonalny rozwój *Hemistomum alatum* Dies. na podstawie badan' cioswiadcuzulnych. Die postembryonale Entwicklung von *Hemistomum alatum* Dies. auf Grund experimenteller Untersuchungen. Extr. du Bull. d. l'Acad. Polon. d. Sci. e. d. Lettres, Cl. d. Sci. Math. e. Nat., Ser. B, Sci. Nat., 1921: 237-250.
- SZIDAT, L. 1923. Beiträge zur Entwicklungsgeschichte der Holostomiden. Zool. Anz., 58: 299-314, 9 text figs.
- 1924. Beiträge zur Entwicklungsgeschichte der Holostomiden. II. Zool. Anz., 59: 249-266, 12 text figs.
- 1929. Beiträge zur Kenntnis der Gattung *Strigea* (Abildg.). Zeitschr. f. Parasitenk., 1: 612-764, 70 text figs.
- TABANGUI, M. A. 1926. Worm Parasites of Chickens. Philippine Agric. Rev., 19: 3-42, 2 pls., 21 text figs.
- THOSS, E. 1897. Über den Bau von *Holostomum cucullus* nov. spec. Dissertation, pp. 1-66, 2 pls.
- VAN HAITSMA, J. P. 1925. *Crassiphiala bulboglossa* N. Gen., N. Spec., a Holostomatid Trematode from the Belted Kingfisher, *Ceryle alcyon* Linn. Trans. Am. Microsc. Soc., 44: 121-131, 2 pls.
- 1930. Studies on the Trematode family Strigeidae (Holostomidae). XXI. Life-Cycle and Description of the Cercaria of *Cotylurus michiganensis* (La Rue). Journ. Parasitol., 16: 224-230, 3 text figures.
- VON LINSTOW, O. 1894. Helminthologische Studien. Jenaische Zeitschr. f. Naturwissensch., 26 (N.F., vol. 21): 328-342, 2 pls.
- WENYON, C. M. 1926. Protozoology. 2 vols. Bailliere, Tindall and Cox. London.

EXPLANATION OF PLATES XLI-XLII

All the figures, except 11 and 12, are drawn according to the same scale indicated by the reference line near Figure 10. The magnification of Figures 11 and 12 is indicated by the reference line near Figure 12. Both reference lines represent 0.1 mm.

ABBREVIATIONS

<i>ac</i> — acetabulum	<i>n</i> — nerve strands
<i>ag</i> — adhesive gland	<i>ofb</i> — opening of forebody
<i>alh</i> — anterior lobe of the holdfast organ	<i>oot</i> — oötype
<i>apex</i> — antero-posterior excretory vessel	<i>os</i> — oral sucker
<i>bc</i> — bursa copulatrix	<i>ov</i> — ovary
<i>cvd</i> — common vitelline duct	<i>ovd</i> — oviduct
<i>dex</i> — dorsal excretory vessel	<i>pbc</i> — primordium of bursa copulatrix
<i>eg</i> — egg	<i>ph</i> — pharynx
<i>ejd</i> — ejaculatory duct	<i>plhf</i> — posterior lobe of holdfast organ
<i>eso</i> — esophagus	<i>sv</i> — seminal vesicle
<i>exp</i> — excretory pore	<i>t₁</i> — anterior testis
<i>exs</i> — excretory space	<i>t₂</i> — posterior testis
<i>ga</i> — genital atrium	<i>tex</i> — transverse excretory vessel
<i>hb</i> — hindbody	<i>ut</i> — uterus
<i>int</i> — intestinal cecum	<i>uta</i> — ascending limb of the uterus
<i>lc</i> — Laurer's canal	<i>utd</i> — descending limb of the uterus
<i>lsc</i> — lateral sucking cup	<i>vd</i> — vas deferens
<i>lvd</i> — left vitelline duct	<i>ve₁</i> — vas efferens of <i>t₁</i>
<i>mdex</i> — median dorsal excretory vessel	<i>ve₂</i> — vas efferens of <i>t₂</i>
<i>mdvex</i> — median dorso-ventral vessel	<i>vex</i> — ventral excretory vessel
<i>mg</i> — Mehlis' gland	<i>vit</i> — vitellaria
<i>mvox</i> — median ventral excretory vessel	<i>vr</i> — vitelline reservoir

PLATE XLI

FIG. 1. Reconstruction of female genital system. Left view. The vitelline reservoir lies on the left of Mehlis' gland.

FIG. 2. Reconstruction of the male genital system. Left view. The vas deferens lies between the two vasa efferentia.

FIGS. 3-9. Posterior views of a consecutive series of cross-sections of an early developmental stage of an adult. Of this series Figure 9 represents the foremost section, cut in the region between the two testes. Figures 3-5 show the bursa copulatrix still in the primordial stage; in them the ventral excretory vessels can be traced forward from the excretory pore. In Figure 6 branches from the ventral excretory vessels extend between the

primordium of the bursa copulatrix and the posterior testis to form the ventral excretory vessels. In Figures 7-9 the ventral excretory vessels are separated from the dorsal vessels by the posterior testis, and the dorsal vessels anastomose with subcuticular excretory spaces. Figures 4-9 also show the vitellaria to be in two fields in this early adult stage of development.

FIG. 10. Diagrammatic reconstruction, left side view, showing digestive and excretory systems and the distribution of vitellaria. The peripheral excretory spaces are not represented.

FIGS. 11-12. The external appearance of *C. flabelliformis*, showing the variability of the forebody.

PLATE XLII

FIGS. 13-16. A consecutive antero-posterior series of reconstructions representing thick cross-sections of the forebody, as seen in anterior view.

FIG. 13. The forebody, looking into the cup, showing the two lappets of the posterior lobe of the holdfast organ.

FIG. 14. Reconstruction of sections between the pharynx and acetabulum. The intestinal ceca diverge anterior to the acetabulum.

FIG. 15. Region of the acetabulum, showing both lobes of the holdfast organ, lateral sucking cups and more conspicuous excretory vessels and spaces.

FIG. 16. Region of the adhesive gland. The intestinal ceca bend ventrad. The dorsal and median dorso-ventral excretory vessels are represented.

FIG. 17. Reconstruction of somewhat oblique sections through the dorsal wall of the forebody, showing the relative positions of the digestive, excretory and nervous systems in this body region.

FIG. 18. Reconstruction of cross-sections of the hindbody anterior to the ovary. Anterior view. The ventral excretory vessels are united by the transverse vessel. The intestinal ceca bend ventrad. The vitellaria are distributed throughout almost the entire section.

FIG. 19. Reconstruction of the middle region of the hindbody. Anterior view. Ovary and anterior testis not shown. The median dorsal excretory vessel divides to form the two dorsal vessels. The relative positions of the oviduct, Laurer's canal, common vitelline reservoir, vas deferens, descending limb of the uterus, and ventral excretory vessels are indicated. The vitellaria are restricted to the ventral half of the section.

FIG. 20. Reconstruction of the region behind the posterior testis. Anterior view, showing relative position of seminal vesicle, ejaculatory pouch and duct, descending uterus and ventral excretory vessels anastomosing with subcuticular excretory spaces. Three lobes of the posterior testis are also shown.

PLATE XLI

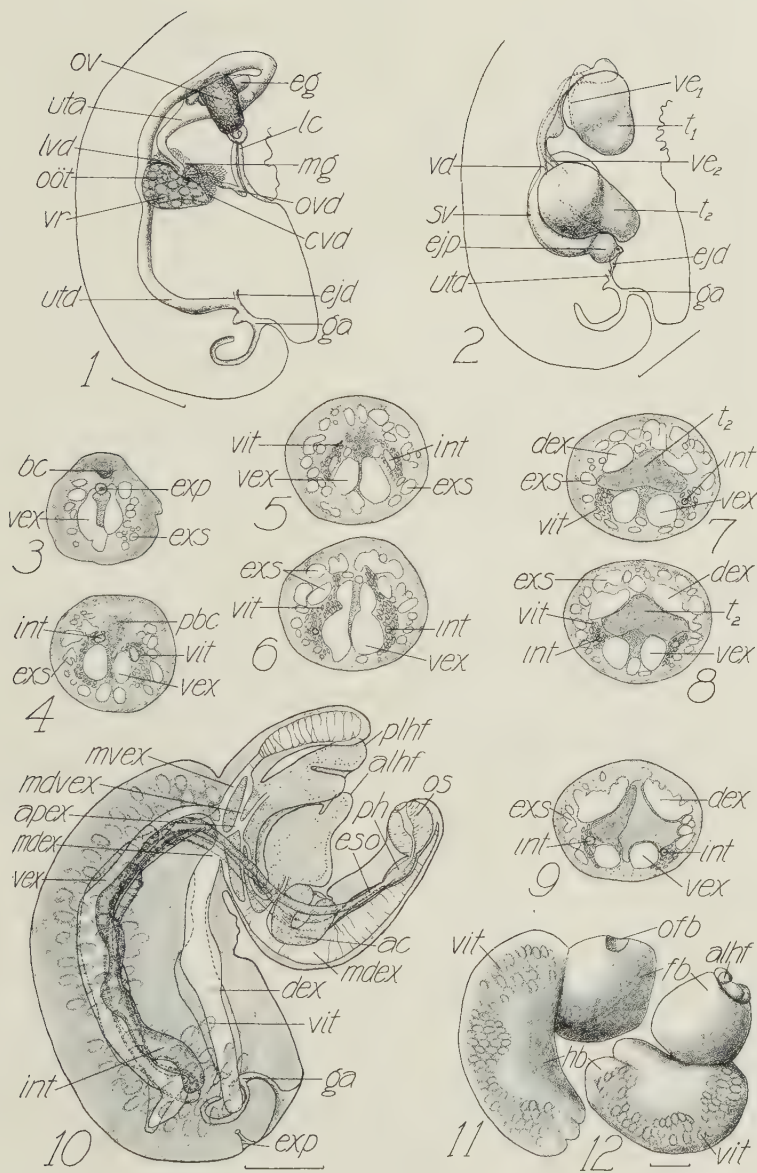
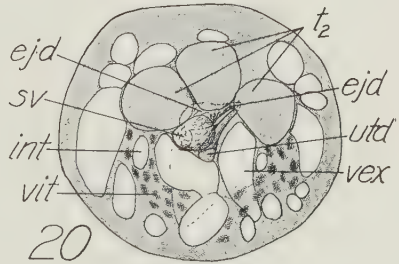
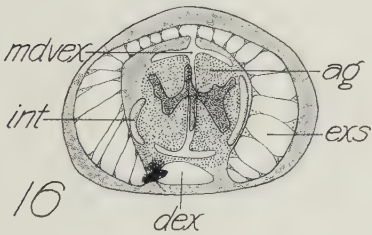
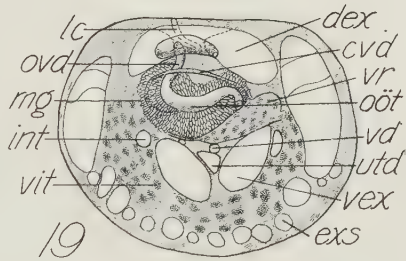
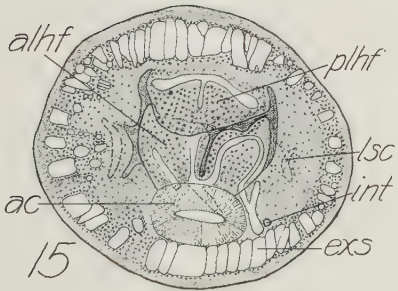
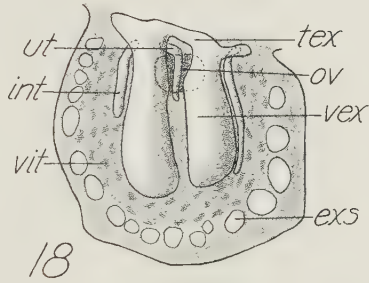
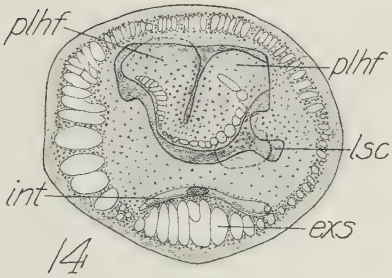
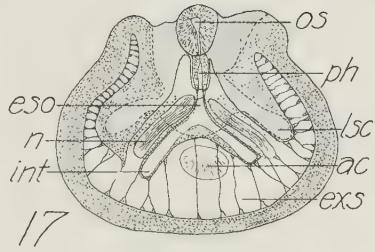
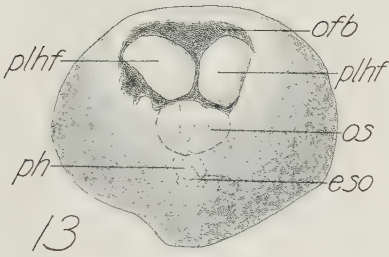


PLATE XLII



STUDIES ON THE TREMATODE FAMILY
STRIGEIDAE (HOLOSTOMIDAE)
NO. XXIII: *DIPLOSTOMUM FLEXICAUDUM*
(CORT & BROOKS) AND STAGES IN
ITS LIFE-HISTORY *

JOHN P. VAN HAITSMA

THE new adult trematode of the family Strigeidae, here described, was obtained as a result of infestation experiments made during the summer of 1927; the life-cycle of this species was experimentally traced during the autumn of the same year. Since, in the course of these experiments, it was shown that the adult develops from *Cercaria flexicauda* Cort & Brooks (1928, pp. 183-186), the name of the present species becomes *Diplostomum flexicaudum* (Cort & Brooks).

The use of the generic name *Diplostomum* for this species requires an explanation. The genus *Diplostomum* was erected by von Nordmann (1832) for certain larval trematodes found in the eyes of certain European fish. The first of these species he described as *Diplostomum volvens*. He erroneously believed his specimens to be adult and gave a detailed description of them accompanied by excellent figures. Later the Ehrhardt brothers, by means of feeding experiments reported by Braun (1894, pp. 156-167), demonstrated that *D. volvens* developed within the laughing gull, *Larus ridibundus*, into *Hemistomum spathaceum* (Rud.). Thus it was shown that the genus *Hemistomum* Dies., 1850, was in part, at least, a synonym of *Diplostomum* von Nordmann, 1832. Krause (1915, p. 233) showed that *Hemistomum*

* Contribution from the Biological Laboratory of Calvin College and from the Biological Station and the Zoölogical Laboratory of the University of Michigan. This is the twenty-third of a series of studies on the family Strigeidae by Professor George R. La Rue, his students and associates.

Dies. is a synonym of *Alaria* Schrank, 1788. Moreover, according to Stiles and Hassall (1912, pp. 88, 281 [*Index Catalog*]), the type of *Hemistomum* Dies., by inclusion and also by first species rule, is *alatum* = *Alaria vulpis*. By reason of this fact the name *Hemistomum* Dies. is a synonym of *Alaria* Schrank, and therefore is not available for those members of the genus *Hemistomum*, 1850, which are non-congeneric with *Alaria alata*. The adult developed from *Diplostomum volvens* does not belong in the genus *Alaria*; therefore the name *Diplostomum* becomes available according to the rules of priority as the generic name for *D. volvens*. *Spathaceum* (Rudolphi, 1819), however, has priority over *volvens* (von Nordmann, 1832). Hence the correct name of von Nordmann's species becomes *Diplostomum spathaceum* (Rud.). Since the present species is congeneric with *D. spathaceum*, it is placed in the genus *Diplostomum* von Nordmann, 1832. *Diplostomum* Brandes, 1888, falls as a homonym, but the group name *Diplostomulum* Brandes, 1892, for the metacercariae continues to be available.

PART I: THE ADULT WORM

Diplostomum flexicaudum (Cort & Brooks) closely resembles two other recently described species of *Diplostomum*. It is very similar in structure to *D. spathaceum* (Rud.), described by Krause (1915, pp. 136-147), and its similarity to *D. huronense* (La Rue), described by La Rue (1926, pp. 26-35), is only slightly less pronounced. All three of these species inhabit the intestines of gulls. Moreover, *D. flexicaudum* and *D. huronense* were obtained from the same species of gulls of the same region, Douglas Lake. Therefore my findings on *D. flexicaudum* are compared in detail with the descriptions of *D. spathaceum* and *D. huronense* referred to above.

SPECIFIC DIAGNOSIS

Diplostomum flexicaudum (Cort & Brooks). — Fixed material, adults: Characters of the genus; forebody, concave ventrally, $1.0-1.4 \times 0.5-0.7$ mm., not set off from the hindbody by a deep groove; hindbody, cylindrical to club-shaped, usually narrower anteriorly, $1.3-1.9 \times 0.37-0.55$ mm.; oral sucker, $0.065-0.08 \times$

0.06–0.085 mm., but smaller than the acetabulum, 0.06–0.09 $\times$ 0.06–0.135 mm., the latter circular to transversely elliptical, often covered by the mushroom-shaped holdfast organ; ovary, ellipsoidal, 0.09–0.135 $\times$ 0.09–0.16 $\times$ 0.13–0.2 mm.; testes horseshoe-shaped, in the posterior half of the hindbody; uterus forming a large coil between the testes; vasa efferentia unite into vas deferens on the anterior surface of the first testis; eggs, 0.09–0.115 $\times$ 0.057–0.07 mm.; habitat, intestines of herring-gulls at Douglas Lake, Michigan. Metacercaria of *Diplostomulum flexicaudum* (Cort & Brooks) = *Diplostomulum gigas* Hughes & Berkhout, 1929; habitat, crystalline lenses of the eyes of common suckers of Douglas Lake. Cercaria of *D. flexicaudum* = *Cercaria flexicauda* Cort & Brooks, 1928.

External features.—The external appearance of *D. flexicaudum*, *D. spathaceum* and *D. huronense* is so similar that the following description of *D. flexicaudum* applies equally well to the other two species. Each animal consists of a ventrally concave forebody, oval in outline, and a more or less curved, club-shaped to cylindrical hindbody (Fig. 1). The anterior end of the forebody is often made irregular by two processes, which extend variably forward on either side of the slightly protruding oral sucker. In other specimens depressions were found at the sides of the oral sucker instead of processes, or a depression may be found on one side and a process on the other in the same specimen. The posterior and lateral margins of the forebody are usually inturned ventrally. The transversely elliptical acetabulum is located near the middle of the ventral surface of the forebody. Behind the acetabulum and often partly covering it is the holdfast organ, mushroom-shaped when extended and usually provided with a more or less deep longitudinal groove on its ventral surface. In the retracted condition the holdfast organ forms a deep cavity extending dorsad and posterad. Owing to its different states of contraction, the dimensions of the holdfast organ differ so greatly that measurements can have no taxonomic significance. The hindbody arises from the posterior dorsal surface of the forebody. The two body regions may be more or less flexed dorsad at their junction, or they may lie in a nearly straight line. On the dorsal side near the

posterior end of the hindbody the circular to elliptical opening of the bursa copulatrix is readily seen.

Measurements. — In Table I dimensions of the body regions and of some of the more regularly shaped organs of *D. flexicaudum* are compared with corresponding measurements of *D. spathaceum* and *D. huronense* made by Krause and La Rue, respectively. In this table Krause's and La Rue's measurements have a much wider range than my own, probably because my measurements were based on a smaller number of specimens, all of which appeared to be full-grown and moderately extended. This may also explain why the mean of my measurements approaches the larger dimensions presented by Krause. If Krause had also recorded the mean of his measurements, it would have greatly facilitated a comparison of the three species. From the present data it appears that all three species are nearly equal in their dimensions. The most notable differences between *D. flexicaudum* and *D. spathaceum* are that *D. flexicaudum* has a somewhat larger ovary and that its eggs are variable in dimensions.

Table I indicates more distinct differences between *D. flexicaudum* and *D. huronense*. The measurements of the forebody, oral sucker and acetabulum of *D. huronense* are larger than my corresponding measurements of *D. flexicaudum*, and the hindbody of *D. huronense* is shorter and thicker, but its pharynx is narrower. The distinctness of these two species morphologically agrees with results which I have obtained from feeding experiments. *D. flexicaudum* was developed from metacercariae found within the lens capsule of the eye of the common sucker, *Catostomus commersonnii* (Lacépède); *D. huronense* was developed from smaller metacercariae found in the vitreous humor of the eye of the trout perch, *Percopsis omiscomaycus* (Walb.). Hughes and Berkhout (1929, pp. 483-494) have described these two metacercariae and pointed out the differences between them.

Organs of attachment. — These organs include the oral sucker, the ear-like lappets or sucker-like depressions at the sides of the oral sucker, the acetabulum, the holdfast organ and the ventrally inturned margins of the forebody. For a description of these organs and an explanation of the sources of the granular secretion

TABLE I: COMPARATIVE MEASUREMENTS OF *D. flexicaudum* (CORT & BROOKS) AND SIMILAR SPECIES

Items	<i>Diplostomum flexicaudum</i>			<i>D. spathaceum</i>	<i>D. huronense</i>		
	Number measured	Range	Mean	Krause's measurements	La Rue's measurements	Mean of La Rue's measurements	
Forebody							
Length.....	13	1.0-1.4 *	1.16	0.60-1.27	0.518-1.33	0.956	
Breadth.....	13	0.5-0.7	0.61	0.36-0.69	0.503-0.873	0.715	
Hindbody							
Length.....	13	1.3-1.9	1.65	0.77-2.1	0.518-1.332	1.038	
Breadth.....	13	0.37-0.55	0.44	0.23-0.71	0.407-0.592	0.490	
Oral sucker							
Length.....	10	0.065-0.08	0.072	0.054-0.094	0.090-0.112	0.099	
Breadth.....	16	0.06-0.085	0.069	0.054-0.099	0.051-0.105	0.079	
Pharynx							
Length.....	10	0.07-0.09	0.077	0.041-0.081	0.075-0.098	0.083	
Breadth.....	6	0.04-0.06	0.05	0.036-0.072	0.036-0.061	0.048	
Acetabulum							
Antero-posterior.....	16	0.06-0.09	0.076	0.054-0.11	0.060-0.12	0.085	
Breadth.....	16	0.06-0.135	0.09	0.059-0.14	0.097-0.158	0.111	
Ovary							
Antero-posterior.....	10	0.09-0.135	0.109	0.077-0.13	0.069-0.148	0.120	
Dorso-ventral.....	10	0.09-0.16	0.121	0.099-0.12			
Breadth.....	10	0.13-0.2	0.165	0.10-0.17	0.123-0.181	0.166	
Egg							
Length.....	14	0.09-0.115	0.101	0.108	0.095-0.105	0.104	
Breadth.....	14	0.057-0.07	0.062	0.063	0.053-0.060	0.057	

* All measurements are given in millimeters. Measurements of the body regions were made upon unmounted specimens. Length was measured along the median line of the respective body regions as seen in ventral view. The breadth measurements represent maximum dimensions as seen in frontal aspect. The measurements of the length of the forebody include the lateral lappets when they were extended. The breadth measurements of the ovary and acetabulum are only approximations. They represent the products of the thickness of the sagittal sections multiplied by their number. My egg measurements were obtained from eggs collected from the feces of the gulls. The egg measurements of *D. huronense* were made upon uterine eggs (La Rue, 1926, p. 30).

in the tips of the ear-like lappets or sucker-like depressions reference may be made to Krause's description (1915, pp. 106-111) of *D. spathaceum*. My observations have yielded additional information concerning the functions of these organs. According to these observations the chief organs of attachment of these parasites are the extended ear-like lappets at the sides of the oral sucker. In a portion of the host intestine, fixed in corrosive-acetic, the parasites were attached only by means of the extended ear-like appendages or lappets which had been pressed into the tissues of the host. In two specimens which had been shaken free from the host finely granular globules, lightly stained with hematoxylin, were found on the secreting surfaces of the lappets. These globules appeared to be mucilaginous. Their presence suggested that the parasites were attached by means of this substance rather than by any mechanical action of the lappets. This explanation agrees with that of von Linstow (1877, pp. 187-198), who called the secreting glands "Leimdrüsen." The columnar epithelium of the host intestine had been completely removed, presumably owing to the post-mortem digestive action of the host, since the denuded area extended beyond the range of the parasites. Consequently, the parasites were attached to the subepithelial connective tissue of the host. In attached parasites, which had been killed and fixed *in situ*, the oral sucker, acetabulum and holdfast organ gaped widely open, but they were not in contact with the tissues of the host. Their gaping condition indicated that the parasites had released their hold before they died. In some specimens shaken free from the host the acetabulum protruded almost entirely beyond the ventral surface of the forebody: It is probable that the oral sucker, acetabulum, holdfast organ and the ventrally inturned margins of the forebody coöperate with the ear-like lappets of the living parasites in the process of attachment. It also seems probable that the lappets and acetabulum are contracted in life so as to hold the ventral surface of the parasite closely against the intestinal wall of the host. In the holdfast organ glandular tissue continuous with that of the so-called adhesive gland extended to the ventral surface. Presumably the secretion of these glands is conveyed into the cavity of the holdfast organ, where

it may perform a function, possibly digestive. In the present material, however, the only basis for this belief is to be found in the position of the glandular cells. The intestinal wall of the host did not reveal serious pathological disturbances which could be ascribed to the presence of the parasites.

Digestive system. — This system of organs of *D. flexicaudum* (Fig. 1) resembles that found in other diplostomes. On being shown some sections of this parasite, Dr. William McKay German, pathologist at Blodgett Hospital, Grand Rapids, Michigan, recognized a brownish pigment in the digestive ceca which he interpreted to indicate old blood. From this it appears that this parasite fed upon the blood of its host. Disintegrating nuclei were also present in the digestive ceca. Injuries to the host tissue which might explain the source of the blood were not observed.

Amphitypy. — Krause described *D. spathaceum* as amphitypic. A similar condition was found in *D. flexicaudum*. The ovary and Mehlis' gland may be found in the right side of some specimens and in the left in others. This reversal of position of these organs is accompanied by a reversal of position of the other organs which do not lie in the median plane and are not bilaterally symmetrical, particularly the ascending limb of the uterus and the vasa efferentia.

Male reproductive system. — The testes of *D. flexicaudum* (Fig. 2) are roughly horseshoe-shaped. They lie in the posterior half of the hindbody. The vasa efferentia arise from the anterior median ventral surfaces of the testes and unite with the vas deferens in front of the first testis (Figs. 2, 5). The vas deferens bends toward the median ventral line and enlarges behind the posterior testis to form the seminal vesicle. The seminal vesicle coils vertically, first extending dorsad and posterad to the genital atrium and then bending anterad to the posterior testis (Figs. 3-4), where its diameter diminishes and its wall becomes thickly muscular to form the ejaculatory duct. The ejaculatory duct extends dorsad and posterad to the genital atrium into which it opens dorsal to the opening of the uterus. The position of the testes and male genital ducts of *D. flexicaudum* agrees in detail with that given in Krause's

description of these organs in *D. spathaceum*, but in *D. huronense* the testes are located relatively farther forward (La Rue, 1926, p. 31, and Fig. 1).

Female genital organs. — From the posterior dorsal side of the ellipsoidal ovary the oviduct extends dorsad and posterad along the dorsal side of the anterior testis to the right or the left of the median line to Mehlis' gland (Fig. 10). A short distance from the ovary the oviduct is slightly enlarged (Fig. 8), but this enlargement is not so definite as Krause's (1915) figure N indicates for *D. spathaceum*. From this enlargement Laurer's canal arises and extends to its opening near the median dorsal line of the hindbody. As the oviduct enters Mehlis' gland the common vitelline duct empties into it (Fig. 6). Closely behind the junction of these two ducts the oötype bends to the right, or to the left, and becomes the ascending limb of the uterus, which begins its "ascent" anterad by forming a large spiral coil between the testes (Fig. 9). Thence the ascending uterus extends anterad along that side of the body containing Mehlis' gland, over the ovary (Fig. 7), to a point somewhat beyond one half of the distance between the ovary and the anterior end of the hindbody (Fig. 10). Here it bends and becomes the descending uterus, which extends along the midventral line ventral to the vas deferens to the genital atrium. The course of the female genital ducts, here described, agrees in detail with that of *D. spathaceum* described by Krause (1915, text fig. N), except that in *D. flexicaudum* the coil of the ascending uterus between the testes is more extensive. The oviduct of *D. flexicaudum* near its junction with Laurer's canal is less coiled than the corresponding part of the oviduct of *D. huronense* as described by La Rue (1926, Fig. 4).

The vitelline system is closely similar in *D. flexicaudum*, *D. spathaceum* and *D. huronense*. In each of these species the vitellaria extend from a position somewhat anterior to the acetabulum to the posterior end of the hindbody. In the region of the testes of all three species the vitellaria form only a narrow midventral strip. The points of entrance of the right and left vitelline ducts into the intertesticular vitelline reservoir shown in my Figure 6 is an individual characteristic. In most specimens of *D. flexicaudum*

the right and left vitelline ducts empty into the ventral end of the vitelline reservoir.

The excretory system. — My findings on the excretory system of *D. flexicaudum* agree quite closely with those described by Krause (1915, pp. 145–146) for *D. spathaceum*, but in the light of my previous studies on the excretory systems of *Crassiphiala bulboglossa* Van Haitsma (1925, p. 124) and of *Cotylurus flabelliformis* (Faust) (Van Haitsma, 1931), my interpretation of the excretory vessels of *D. flexicaudum* is somewhat different.

The excretory system can be traced most easily by means of cross-sections of an early developmental stage of the adult (Figs. 11–19). The common excretory duct, opening at the excretory pore posterior to the opening of the bursa copulatrix, is short, its length being equal to the thickness of the body wall (Fig. 19). The common excretory duct divides into two branches, which extend ventrad along either side of the bend of the descending limb of the uterus near its posterior end (Fig. 19). Near the ventral side of the body these two branches bend anterad and continue along either side of the descending limb of the uterus throughout its length (Figs. 14–19). These right and left ventral excretory ducts are the “Uterusgefäße” of Krause. In front of the anterior bend of the uterus they unite into a large median ventral vessel which extends to the posterior part of the forebody (Fig. 13), where it anastomoses with numerous small excretory canals found in the ventral lip of the forebody. These canals continue anterad in the lateral regions of the forebody (Figs. 11–12).

From the dorsal region of each of the two branches of the common excretory duct (Figs. 18–19) a dorsal vessel arches around the side of the bursa copulatrix and opens into wide peripheral excretory spaces of the dorsal region and sides of the posterior half of the hindbody (Figs. 16–17). For this reason the right and left dorsal vessels of this region of the hindbody are not distinguishable from the peripheral excretory spaces. In the region of Laurer’s canal the right and left dorsal vessels again appear distinctly (Fig. 15), and at a short distance in front of this canal they unite into a median dorsal vessel that extends to the anterior

end of the hindbody (Fig. 14). Immediately behind the adhesive gland at the junction of forebody and hindbody the median dorsal and median ventral vessels unite into a vessel that connects with numerous small canals in the ventral lip of the forebody, as already explained (Fig. 13).

In the forebody there are, in addition to a median vessel, three lateral vessels on each side. One of them lies dorsal to the digestive cecum; another is found near the nerve strand; the outermost one is located near the margin of the forebody (Figs. 11-12). These lateral vessels of the forebody are small and sometimes difficult to distinguish in preserved specimens. At the level of the pharynx they form on each side a close network which anastomoses with the median vessel. The median vessel extends posterad to the region dorsal to the holdfast organ (Figs. 11-12), where it is dilated. Behind the holdfast organ it is connected with the lateral vessels by means of transverse vessels (Fig. 13). It appears probable that the median vessel of the forebody is also connected by means of small indistinct canals with the median dorsal vessel of the hindbody.

This interpretation of the excretory system of *D. flexicaudum* is in complete accord with my findings on the excretory systems of *Crassiphiala bulboglossa* Van Haitsma (1925) and *Cotylurus flabelliformis* (Faust) (Van Haitsma, 1931) as far as they were traced, except that the dorsal vessels of the posterior half of the hindbody of *D. flexicaudum* are indistinct, owing to their anastomoses with peripheral excretory spaces. Although several previous investigators (Brandes, 1890, pp. 568-570; Poirier, 1886; Guberlet, 1922) have recognized only two main excretory vessels in the hindbodies of this group of parasites, it is believed that the interpretation of the excretory system of the hindbody here presented will be found applicable to the excretory systems of the hindbodies of all adult Strigeidae.

Pathological symptoms in the definitive hosts. — As explained above, no pathological effects of the parasites were observed in the tissues of the hosts, but there were indications that the parasites fed upon the blood of the host. Heavy infestations appeared to retard the growth of the gulls and to keep them in an emaciated

condition even though they were well fed. Weakness and nervousness were also indicated by the movements of the wings of the birds. In birds heavily infested the wings drooped repeatedly, only to be drawn back spasmodically. Such movements were continually observed in my gulls for about two weeks before they were killed. It can only be conjectured whether the movements were caused by secretions of the parasites or by the mechanical irritation of the parasites upon the intestinal wall of the host. These pathological symptoms are similar to some observed by Van Haitisma (1931) in domestic ducks heavily infested with *Cotylurus flabelliformis* (Faust).

Comparison of D. flexicaudum and D. spathaceum. — Owing to the close resemblance in structure and life-history between *D. flexicaudum* and *D. spathaceum*, as the latter was described by Krause (1915) and Szidat (1924), it is desirable to summarize the differences between them. The dimensions of the eggs of *D. flexicaudum* are $0.09\text{--}0.115 \times 0.57\text{--}0.07$ mm. According to Krause, the measurements of the eggs of *D. spathaceum* are invariably 0.108×0.063 mm. According to evidence which will be presented in a later article, the miracidium of *D. flexicaudum* was experimentally developed in *Lymnea emarginata angulata*, but, according to Szidat, the miracidium of *D. spathaceum* develops in *L. stagnalis*, *L. palustris* O. F. Müll. and *L. ovata* Drap. The cercaria of *D. flexicaudum* assumes a characteristic resting position in the water. With the tail furcae outspread upwardly and with the tail-stem bent, the body hangs in the water in a nearly horizontal position. Szidat (1924, p. 251, and Fig. 1) describes a different resting position for the cercaria of *D. spathaceum*. I found the diplostomula of *D. flexicaudum* in the lenses of the eyes of common suckers of North America; Szidat found the diplostomula of *D. spathaceum* in the eyes of *Acerina cernua* and *Leuciscus rutilus*, and von Nordmann found it in the vitreous humor and the lens of *Perca fluviatilis* and the burbot (*Gadus lota*), all in Europe. The adults of these two species also differ in several minor ways. In *D. flexicaudum* the pharynx averages slightly longer than the oral sucker and it is almost as wide. According to Krause, the pharynx of *D. spathaceum* is nearly always shorter than the oral sucker and less than

three fourths as wide. In *D. spathaceum* the acetabulum is seldom covered by the holdfast organ, but according to my findings this is often the case in *D. flexicaudum*. It also appears from my measurements of Table I that the ovary of *D. flexicaudum* is somewhat larger in proportion to the size of the body than the ovary of *D. spathaceum*. Then, too, the coils of the uterus between the testes appear to be larger in *D. flexicaudum* than in *D. spathaceum*. In view of all these differences it appears that *D. flexicaudum* and *D. spathaceum* are distinct species, but, owing to the many close similarities between them, it is desirable that a careful comparison of their structure and life-histories should be made to prove that the differences which I have found are not due to differences in observation. In this connection it is of interest that common terns banded in America have later been found in Europe.

PART II: LIFE-HISTORY

The life-history of *Diplostomum flexicaudum* (Cort & Brooks) was the third American strigeid life-cycle to be determined during the summer and autumn of 1927. Infestation experiments here described have demonstrated that the adult of this species developed from *Diplostomulum gigas* Hughes & Berkhout (1929, pp. 483-488) and that this metacercaria in turn developed from *Cercaria flexicauda* Cort & Brooks (1928, pp. 183-186). The taxonomy of this species has been discussed in connection with the description of the adult, but the identification and naming really depended upon a knowledge of the life-cycle, which was obtained by means of the following infestation experiments.

Metacercaria to adult. — Considerations of an *a priori* nature suggested that some of the heavy experimental parasitic infestations of the herring-gulls of Douglas Lake region were obtained from the common suckers of this lake. To test this suggestion common suckers, *Catostomus commersonnii* (Lacépède), were fed to two nestling herring-gulls, *Larus argentatus* (Pont.), during the summer of 1926. A post-mortem examination of these birds revealed that the intestines of each contained more than one hundred individuals of a species of *Diplostomum* in all stages of develop-

ment from metacercariae to sexually mature adults. Since both gulls had been obtained from their nesting grounds on June 29, when they were only about one week old, and thereafter had been fed during seven weeks only on cooked fish and the suckers which were given them for experimental purposes, these feeding experiments indicated clearly that the common suckers were the source of the infestation. Previous experiments by the Ehrhardt brothers (Braun, 1894, pp. 165-167) had demonstrated that *Diplostomum spathaceum* (Rud.) developed from a diplostomulum (*Diplostomum volvens* von Nordmann) in Europe. It was presumed, therefore, that *Diplostomum flexicaudum* also developed from a diplostomulum. La Rue, Butler and Berkhout (1926, pp. 285-286) had already pointed out that the crystalline lenses of the eyes of more than 94 per cent of the common suckers of Douglas Lake were heavily infested with metacercariae, which averaged more than thirty in each eye. Hughes and Berkhout (1929, pp. 483-486), after a careful study of these metacercariae, considered them all to belong to one species of diplostomulum, and my observations led me to accept their interpretation. Therefore it was concluded from the experiments of 1926 that the diplostomulum of the eyes of the common sucker of Douglas Lake developed into the adult *Diplostomum* found in my experimental herring-gulls.

This conclusion, drawn from the experiments of 1926, was carefully tested during the summer of 1927. Two herring-gulls about one week old were obtained from their nesting grounds and kept in cages where their food was under complete control, except for insects which might enter the cages. The birds were fed cooked fish and occasionally pieces of fresh beef. The water supplied to them was pumped from a well. In the course of the experiment one hundred and thirty-nine eyes of common suckers were fed to the two gulls between July 9 and August 8. One of the birds was killed on August 8, and the other on August 13. They were carefully examined for their parasites and each was found to contain more than two hundred *Diplostomum flexicaudum* in various stages of development. The gull killed first contained some individuals developed only slightly beyond the diplostomulum stage. The uniform results of these repeated experiments established

beyond doubt that the diplostomulum of the eye of the common sucker of Douglas Lake is the metacercaria of *Diplostomum flexicaudum*.

The eggs. — On August 8, just before the first gull was killed, several hundred eggs were collected from the feces of both gulls. The eggs, carefully separated from all solid matter and repeatedly washed in clean water, were kept in well water in labeled watch glasses. The development of miracidia in these eggs was observed from time to time, but was not carefully studied. Since all the strigeids, *sensu lato*, found at the autopsy of these gulls belonged to this one species, there is no doubt that these eggs also belonged to the same species.

Miracidia. — Miracidia were first observed in the dishes containing the eggs on August 27, nineteen days after the eggs were collected, and thereafter they appeared daily for about two weeks. They hatched in larger numbers during the morning than during afternoon or night. The explanation for this phenomenon may be found in the method of hatching of the miracidium. The loosening of the operculum of the egg is probably a chemical, rather than a mechanical, process, depending upon a secretion of the miracidium rather than upon the pressure exerted by the miracidium. Barlow (1925, p. 24) discussed such an explanation for the hatching of the miracidium of *Fasciolopsis buski* and it seems to me to be a plausible explanation for the hatching of this miracidium. The rate of development doubtless varies directly with the temperature and consequently development continues more slowly during the night, but hatching is apparently delayed by darkness, possibly because light stimulates the production of the secretion employed in loosening the operculum. These miracidia are positively phototactic toward diffused light. In a darkened room they appeared to show some preference for the dimly lighted side of a watch glass. They usually swim near the surface of the water. In this stratum the snails into which they penetrate are also found in largest numbers during the daytime.

A reason for believing that the eggs from which these miracidia developed all belonged to one species has already been stated. An additional reason for this belief may now be given, for a detailed

study showed that the miracidia were similar in all respects. They are described below.

Infestation experiments with miracidia upon snails. — In eight infestation experiments from August 31 to September 6 the following snails were exposed to these miracidia, or miracidia and eggs: *Planorbis trivolvis* Say, *Lymnea stagnalis perampla* Walker, *L. lanceata* Gould, *L. emarginata angulata* Sowerby, *Physa parkeri* "Currier" De Camp, *Physa gyrina* Say and *Planorbis campanulatus* Say. These snails, most of them about half-grown, had been collected from Douglas Lake more than three weeks before the infestation experiments began and during that time they had been kept in aquaria in the laboratory. Here infestation with miracidia was impossible, for they were kept in well water and were fed lettuce which had been grown far from the lake. In each of the eight experiments miracidia were placed in water in watch glasses together with a specimen of several species of the snails named above and they were observed under a dissecting microscope, but in no case was the actual penetration of a miracidium into a snail observed. Thereafter the miracidia, together with eggs in some instances, and the snails were kept in labeled bottles where the snails were cared for in the same way as they had been in the aquarium.

Miracidia to cercariae. — On October 15 and 16 three of the eight experiments involving miracidia of *Diplostomulum flexicaudum* and the snails yielded positive results, since one snail of each of these three experiments produced cercariae. In one of these experiments the snails had been exposed to three miracidia and twenty embryonated eggs on August 31, and in each of the other two experiments, to twelve miracidia on September 1. It is noteworthy that the weather was unusually warm during the two days when these infestation experiments were performed and that the number of miracidia which hatched was greatest during that time. The miracidia also appeared to be extraordinarily active. In the three experiments yielding positive results all of the seven species of snails named above were represented, but the cercariae came only from three specimens of *Lymnea emarginata angulata* Sowerby. A careful, detailed study of these cercariae showed

that they were similar in all respects and consequently that they belonged to one species. Professor W. W. Cort confirmed my identification of them as *Cercaria flexicauda* Cort & Brooks, 1928. Three kinds of evidence warrant the conclusion that *Cercaria flexicauda*, obtained in three of my experiments, resulted from my experimental infestation of the snails with miracidia. The water in which the snails exposed to the miracidia were kept was carefully watched for the appearance of cercariae, but none were found in it until forty-five days after the experiments. This period agrees with the results of the experiments of Mathias (1925, p. 63) upon *Strigea tarda* (Steenstr.), in which a corresponding development required six weeks, and also with the result of my experiment (Van Haitsma, 1930) upon *Cotylurus michiganensis* (La Rue), in which the time of development from miracidia to cercariae was forty days. The alternative that my snails were already infested with miracidia when they were collected from Douglas Lake would imply that the development of *Cercaria flexicauda* from miracidia during the warmest season of the year required more than sixty days, a conclusion that seems unreasonable. The fact that all three of my experiments were consonant in all respects also shows clearly that *Cercaria flexicauda* resulted from my experimental infestation of *Lymnea emarginata angulata* with miracidia. Moreover, the later described infestation experiments with *Cercaria flexicauda* on suckers demonstrated that these cercariae continued their development in the crystalline lens. Therefore it is concluded that *Cercaria flexicauda* is the larva of the Diplostomum obtained by me from the herring-gull.

Snail host of the miracidium. — From the fact that I obtained *Cercaria flexicauda* from *Lymnea emarginata angulata* only, it should not be inferred that this species of snail is the only possible host of this cercaria. Cort and Brooks (1928, pp. 183–186) obtained it from the following snails: *Lymnea emarginata angulata* Sowerby, *L. stagnalis appressa* Say, *L. stagnalis per ampla* Walker and *L. humilis modicella* Say. It is not clear why only one species of *Lymnea* proved to have become infested with miracidia in my experiments, since other species of this genus of snails were exposed to the miracidia at the same time. Several of my exposed snails

died during the experiments. It may be that some of them died owing to unobserved infestations. During my experiments no definite response of the miracidium of *Diplostomum flexicaudum* to the presence of any species of snail was observed. For these reasons I do not infer such a definite host specificity for this miracidium as the results of my experiments seem to indicate.

Infestation experiments with cercariae upon suckers. — In order to complete this life-cycle experimentally three infestation experiments were performed with cercariae of *D. flexicaudum* on suckers by pouring small amounts of water containing the cercariae into the aquaria containing the fishes. These experiments demonstrated that the cercariae penetrated the fishes and severely irritated them during the penetration. In all of my experiments the suckers died too soon after the penetration of the cercariae to permit the metacercariae to develop fully. Post-mortem examination of the fishes revealed large numbers of partially developed metacercariae in their eyes, and some within the lens capsule. Since the cercariae penetrate all parts of the bodies of the fishes, the experiments demonstrate clearly that the metacercariae normally inhabit the crystalline lens and to this extent present evidence for the genetic connection of the stages of this life-history already mentioned. These experiments will be described in greater detail below in connection with the discussion of the cercaria. Reasons will also be presented there for the belief that this parasite is the cause of numerous deaths of fishes in nature and in breeding ponds, and that it is therefore of considerable economic significance.

THE MIRACIDIUM

Form and measurements. — This miracidium is fusiform, widest about one third of its length from the pointed anterior end, and roundly truncate posteriorly. Its outline is nearly circular in anterior view. A moderately extended specimen measured under a cover-glass is about 0.17 mm. long and 0.04 mm. wide at the level of the two conspicuous eyespots (Fig. 24). The eyespots are normally found in the region of the greatest width. The shape and measurements of the miracidium may differ very much in

different contraction states. In a greatly contracted specimen the length may be one half as great and the width may be almost twice as great as the corresponding measurements of a moderately extended specimen.

Movements. — The movements of the miracidia of *Diplostomum flexicaudum* are more even and appear to be more purposeful than those of *Paramoecium*. Barlow's (1925, p. 27) description of the movements of the miracidia of *Fasciolopsis buski* (Lankester) are applicable here, for the present miracidia also swim about as if they were eagerly seeking something. Although these miracidia can back away from solid objects, their movements are usually forward. Ordinarily when they are swimming in a watch glass their eyespots remain on the upper side, but occasionally one may be observed to roll about on its long axis without changing its rate of progress. At times, a miracidium may cease its forward movement and employing either its anterior or its posterior end as a pivot may spin about so that its movement circumscribes a cone. If a miracidium under a cover-glass comes in contact with an air bubble, it may slowly glide along the surface of the bubble while constantly attempting to penetrate the surface film with its protrusile anterior end. Similar penetrating movements have also been observed in miracidia which came in contact with a slip of paper employed to hold up the cover-glass. The actual movements of penetration of a miracidium of this species into a snail have not been observed.

Cilia. — These miracidia swim by means of cilia which are about ten micra in length. The cilia completely cover the body except at the anterior tip and at the two conspicuous lateral processes found on each side of the body immediately in front of the level of the eyespots and between the epidermal plates. On the dorsal surface of the miracidium of *Strigea tarda* Mathias found a rectangular area, free from cilia, directly above the eyespots. Such a non-ciliated area was not found in the present miracidium. Ciliary movements are maintained unceasingly as long as the animal is alive. Even if the anterior part of the body is ruptured so that its cells flow out, the cilia on the posterior part of the organism may still continue to beat for a long time.

Eyespots and nervous system. — The most conspicuous organs of the miracidium are a pair of dark brown, almost black, eyespots, which are located dorsally and laterally in the region of greatest width. They have the form of thick hemispherical bowls and are composed of numerous spherical, melanotic granules. From the laterally directed concavity of each eyespot two lenses protrude outward and forward. Underneath the eyespots there is a mass of comparatively large spherical cells with granular contents. The extent of this mass of cells appeared to vary in different specimens and its outline was difficult to determine, but numerous attempts to determine its shape led to the conclusion that it is bilobed (Fig. 24). Cort (1919, pp. 511–516) represents the nervous systems of the miracidia of *Schistosoma mansoni* and *Sch. japonicum* as oval to quadrangular, and Sewell (1922, p. 285) figures the nervous systems of the miracidia of *Sch. haematobium* and of *Cercaria Indicae* XV as circular in outline. Mathias (1925, p. 37) describes the nervous system of the miracidium of *Strigea tarda* as circular in dorsal view, while Barlow (1925, p. 23) represents this organ as irregularly rectangular in the miracidium of *Fasciolopsis buski*. These differences in the descriptions of the nervous system are probably in part indicative of the difficulty experienced in determining its outline. Nerve filaments proceeding from its cells, such as Barlow (1925, p. 23) observed to extend forward and backward from this organ in the miracidium of *Fasciolopsis buski*, were not seen in the miracidium of *D. flexicaudum*. Faust (1924, p. 28, and Fig. 11) has described the central neural mass of *Schistosoma japonicum* as circular in outline in dorsal view, but his Figure 11 also shows masses of gland cells at the sides of the neural mass, so that the neural mass and the masses of gland cells together have a more bilobed shape. I was not able to distinguish gland cells as described by Faust. In fact his observations on this point have not been confirmed by other workers.

Primordial digestive organ. — Extending along the median region of the anterior part of the body to its tip there is a clavate rudiment of a digestive system which is composed of large cells. In some specimens this club-shaped organ seemed to extend ventral and posterior to the brain, but its extent posteriorly could not be

definitely determined. In the anterior part of the body this organ was more distinctly visible and it appeared to be more rigid than the surrounding tissue. This rigidity is doubtless due to the turgidity of its cells. In keeping with its appearance of greater rigidity the protoplasm of its cells was more densely granular. In the present miracidium there were slight indications of an internal canal in this organ. A similar hollow digestive organ was reported for the miracidia described by Sewell, Mathias and Barlow. Although this may be a rudimentary digestive organ, as is generally supposed, its position and rigidity suggest that it also performs an important function during the penetration of the miracidium into a snail. "Salivary glands," such as Sewell described for the miracidia of *Sch. haematobium* and *Cercaria Indicae* XV, could not be definitely discerned.

Excretory system.—As in other Strigeata (La Rue, 1926, p. 276), there are two pairs of flame cells in the miracidium of *D. flexicaudum* (Fig. 24). The anterior pair is located near the dorsal surface a short distance behind the eyespots and the posterior pair is found in the lateral posterior region, between the third and fourth quarters of the body. The flame cells are large in proportion to the size of the body and their flickering appearance becomes more distinctly visible as the water under the cover-glass is diminished. The proto-nephridial systems of the two sides of the body are not connected with each other. On each side of the body capillaries extend from the anterior and posterior flame cells to a point about half-way between them, where they unite to form a main collecting tubule. From its point of origin each main tubule extends forward almost to the eyespot on the same side of the body. Here the main tubule bends posterad and continues almost directly to the region of the posterior flame cell where, after forming a double coil, it arches forward and outward to the excretory pore. Each excretory pore opens to the outside laterally near the level of the posterior flame cells. Between the organs of the interior and the body wall of the miracidium there is a space in which the main excretory tubules are dilated into little ampulliform bladders just before they open through the excretory pores. These bladders are closely similar to those observed in the furcae

of the cercariae of this species. Since the internal organs are attached to the body wall at only a few points, especially at the anterior and posterior ends of the miracidium, and since these internal organs are independently contractile, there is an almost constant shifting of the relative position of the organs with reference to points on the body wall. Thus the posterior flame cells may be found either before or behind the openings of the excretory pores. The internal organs may also shift their positions with reference to each other. So, for example, the posterior flame cells may point mesad instead of posterad. This shifting of the internal organs and the changes in their shape make difficult the accurate detailed description of a typical miracidium.

Epidermal plates. — The cilia covering the miracidium project from epidermal plates, arranged in four transverse tiers (Fig. 20). The plates of the two anterior tiers could readily be distinguished in moribund specimens, but those of the two posterior tiers were more difficult to distinguish. These plates, as seen in optical section, appear to have thick bands somewhat resembling cell walls extending from their external to their internal surfaces (Figs. 21–23). The studies of Thomas (1883), Schubman (1905) and Ortmann (1908) upon the embryological origin of the plates and of Coe (1896) on their structure have shown that each plate represents a single cell. Nuclei were not seen. In moribund specimens the ectodermal plates were sometimes sloughed off severally, each as a unit. Only in this way was their number in the two posterior tiers determined. In a dorsal view the foremost tier showed three plates, the second four, the third two, and the fourth one and one half. If one assumes that the dorsal and ventral surfaces are alike with respect to the number of plates, there are twenty-one plates in all, the same number which Mathias (1925, p. 38) found in the miracidium of *Strigea tarda*. The boundary between the first and second tiers of plates is found immediately in front of the eyes; that between the second and third tiers is located near the middle of the body; and that between the third and fourth tiers crosses the miracidium immediately behind the excretory pores.

Lateral processes. — Lateral processes similar to the larger

lateral processes of this miracidium (Figs. 20-24) have been represented for the miracidia of *Sch. mansoni* and *Sch. japonicum* by Cort (1919, pp. 511-516) and of *Sch. haematobium* and *Cercaria Indicae* XV by Sewell (1922, p. 285), but both these investigators represent only one process on each side of their respective miracidia. Cort called these processes anterior ducts. Sewell (1922, p. 287) believed that the similar processes of the miracidia of *Cercaria Indicae* XV and *Sch. haematobium* are connected with excretory canals which he supposed to lie between the ectodermal plates of the first two tiers.

In the miracidium of *D. flexicaudum* there are two lateral processes in series on each side and situated at the level of the boundary between the two anterior tiers of plates. The posterior process is always the larger. The cuticle of this lateral process is thin and is bulged outward. According to Faust (1924, p. 28), similar processes in the miracidium of *Sch. japonicum* represent the ends of ducts carrying to the outside a mucoid secretion from glands lying at the sides of the central nerve cell mass, but such an explanation for the larger lateral processes of the present miracidium was not indicated by my observations. The smaller anterior process on either side appears to be a thin outwardly bulged portion of the cuticle of an epidermal plate.

Inasmuch as lateral processes have been observed in the miracidia of three genera of the Strigeidae, viz., *Diplostomum*, *Cotylurus* and *Crassiphiala*, as well as in the miracidia mentioned above and described by Cort, Sewell and Faust, it is remarkable that Mathias (1925, p. 37) did not find such processes in the miracidium of *Strigea tarda*. Mathias showed a projecting ring of tissue encircling his miracidium immediately in front of the eyespots. Such a ring was not observed in my miracidia. Possibly Mathias misinterpreted these processes as optical sections of a ring. The lateral processes of the miracidium of *D. flexicaudum* are found only at the sides of the body, as was evident from an anterior view and also from the appearance of the rotating miracidium.

Primordial germ cells. — In the posterior end of the miracidium of *D. flexicaudum* a variable number of large granular cells are arranged in a longer or shorter row which forks anteriorly. Cor-

responding cells in the miracidium of *Sch. mansoni* as described by Cort (1919, pp. 511-516), and of *Sch. haematobium* and of *Cercaria Indicae* XV described by Sewell (1922, pp. 285-287), have been interpreted to be primordial germ cells. The foremost of these cells may be separate from the rest, become rounded and lie free in the miracidium. Mathias did not describe similar cells in the miracidium of *Strigea tarda*. The interpretation of these cells is of considerable interest, for it involves the explanation of the method of formation of the daughter sporocysts. Investigators are agreed that miracidia upon penetration into their proper snail hosts develop into mother sporocysts, but opinion is divided concerning the method of formation of the daughter sporocysts (Ssinitzin, 1909, pp. 664-682; Mathias, 1925, p. 45).

THE SPORO CYST

Description and contents. — On December 17, 1927, a snail which had been producing cercariae for more than two months was examined for its sporocysts. Upon removal of the shell of the snail it was observed that its digestive gland had a mottled appearance due to the fact that it was riddled with sporocysts. The color of the sporocysts is pale yellow, lighter than the color of the digestive gland which is brown. The sporocysts are long, unbranched, thread-like tubes (Fig. 26). In living material their walls appear thin and granular. They are so delicate that they could not be separated from the digestive gland of the snail without breaking. The largest pieces which were freed from the snail were almost 1 mm. long. Each piece was constricted more or less at irregular intervals and bore some resemblance to a string of sausages. On account of the constrictions the diameter of a sporocyst may be twice as great in one place as in another. A large sporocyst measured about 0.1 mm. in diameter in the inter-node between the constrictions. From unbroken ends of pieces of sporocysts it could be determined that one end of a sporocyst is closed and rounded, and that the opposite end contains a narrow birth-pore (Fig. 25), so narrow that the cercariae must enlarge its opening considerably in order to escape through it. Figure 25

shows that the birth-pore is not exactly in the center of the end of the sporocyst. In cross-sections it appeared that the wall of the sporocyst was thin in some places where it consisted of only one subcuticular layer, and in other places thicker where the cuticle was lined with several layers of cells. Within the sporocysts were found large spherical cells with conspicuous nuclei, cell masses varying in shape from spherical to ovoid, and fully developed cercariae (Fig. 26). In other parts of the sporocyst a fibrous network with small scattered nuclei was observed. The large cells were probably ova; the cell masses represent germ balls developing into cercariae.

THE CERCARIAL STAGE

Identification. — Cercariae of this species were obtained October 15 and 16, 1927, from three specimens of *Lymnea emarginata angulata* Sowerby, from which they escaped into the water. They were carefully studied and tentatively identified as *Cercaria flexicauda* Cort & Brooks, 1928. My description, together with some specimens fixed and preserved in hot 5 per cent formalin and others stained and mounted on slides, was sent to Professor W. W. Cort, who confirmed my identification. For a description of this cercaria the reader is referred to the work of Cort and Brooks (1928, pp. 183-186).

Infestation experiments. — Two hog-nosed suckers, *Hypentelium nigricans* Le Sueur, which had been caught in the Huron River near Ann Arbor and kept in an aquarium in the laboratory for five weeks, were exposed to these cercariae on October 19. That the cercariae penetrated them was evident from the actions of these normally sluggish fishes. They became exceedingly active and swam about in the aquarium in a spasmodic erratic fashion. Sometimes they also made quick oscillating movements from side to side as if to brush away a source of irritation. At other times they frantically leaped from the water. Four days after the exposure to the cercariae it was discovered that they were severely infested with a protozoan parasite, *Ichthyophthirius*. Treatment to kill the *Ichthyophthirius* also killed the fishes. Thereupon the eyes of one of the fish were examined and more than one hundred

developing metacercariae were found in them. That these were the same species of cercariae to which the fish had been exposed was clearly shown by the seven rows of spines on the spiny cap at the anterior end. No well-developed specimens of diplostomulum were found. The presence of these young metacercariae in the eyes demonstrates conclusively that this cercaria is a form which normally lives in the eye.

Another hog-nosed sucker, about 5 inches long, which had been kept in the laboratory for one week, was infested with the same species of cercariae on November 3, 7 and 10, 1927. The fish reacted to the penetrating cercariae as the two previously described hog-nosed suckers had done. At times it leaped entirely out of the water, just as fishes have often been observed to do in lakes. Probably the similar movements of fishes in lakes are also due in part to the penetration of cercariae. The eyes of this fish appeared bloodshot on November 7 with a wide blood-colored ring around each pupil. Between this red ring and the pupil there was a narrower pale yellow ring. A red streak probably due to congestion of blood was also noted at the base of each pectoral fin. These symptoms remained until November 11 when the fish was found dead at the bottom of the aquarium. At that time the eyes, which noticeably protruded from the head, were removed for examination. Under a lens parasites could be seen through the black pupil as whitish flakes. Attempts to tear open the eyeballs revealed that they were unusually turgid and hard, a condition resembling glaucoma. The choroid coat of the eye was congested with blood. About one hundred cercariae were collected from the eyes and fixed in corrosive-acetic at about 80° C. for future study. No fully developed metacercariae were found. The metacercariae appeared to be in about the same stage of development as in the two previously described hog-nosed suckers, but some of them had already entered the crystalline lens.

A common sucker, *Catostomus commersonnii* (Lacépède), about four inches long, which had been kept in the laboratory about one month, was exposed to the cercariae of *D. flexicaudum* on December 14. The responses of the common sucker to the penetration of these cercariae were similar to those of the hog-nosed

suckers described above. On the following day, however, this fish was found dead on the bottom of the aquarium.

Pathological effects upon fishes. — There is no doubt that the penetration of large numbers of the cercariae of *D. flexicaudum* (Cort & Brooks) into both species of suckers of the two experiments last described was the cause of the death of these fishes. The results of my experiments agree with the experience of Blochmann (1910, pp. 47-49), who accidentally placed eight specimens of *Lymnea stagnalis* in an aquarium with several fishes. The next morning all the fishes in his aquarium were dead. Examination showed that furcocercous cercariae, *Cercaria fissicauda* La Val., were escaping from the snails. In order to determine whether these cercariae caused the death of his fishes he performed several infestation experiments with *Cercaria fissicauda* on fishes of different genera and also on salamander larvae. In every experiment the result of the experimental infestation was the death of the vertebrate. By careful experiments he eliminated other possible explanations than cercarial infestation for the death of his fishes. He also performed an experiment which indicated that the death of his fishes was not due to toxins produced by the cercariae and therefore he supposed that mechanical injuries inflicted by cercariae upon their hosts were the causes of death. Blochmann examined the blood and brain of an experimentally infested fish and found cercariae in them as well as in other tissues. From his observations he concluded that the movements of the cercariae through the tissues, particularly of the vascular and nervous systems, were the most probable causes of the death of his fishes.

The reactions of Blochmann's fishes to the penetration of cercariae were similar to those which I have observed in my experimental fishes, but mine did not die so quickly, probably because my cercarial infestations were not so heavy. Owing to the fact that Blochmann's fishes died very quickly he did not observe such marked symptoms of infestation as I did.

Economic significance. — These experiments indicate that fishes in breeding ponds and in nature which swim into areas inhabited by snails producing strigeid cercariae may be killed by the penetration of large numbers of these cercariae. It is believed that

of the thousands of fishes which are at times washed up on our lake shores every summer the deaths of a considerable percentage are due to mass infestations of furcocercous cercariae. Szidat (1927, pp. 83-90), who made definite observations on the causes of the death of fishes in the Kurischen Haff in connection with his life-history study of *Diplostomum spathaceum* (Rud.), concluded that the cercariae of this species in European lakes caused the death of thousands of *Acerina cernua* and *Leuciscus rutilus*. Although I have not made a careful study of the causes of the death of fishes at Douglas Lake, my observations on fishes in nature support the belief that large numbers are killed by strigeid cercariae in this country also. I have seen epidemic deaths of fishes under conditions similar to those observed by Szidat. Some of these fishes showed bloody spots and streaks and abnormally congested bulging eyes, just as Szidat described. Consequently, I too conclude that the cercariae of *D. flexicaudum* cause the death of a large proportion of the thousands of fishes which are found on the shores of Douglas Lake every summer. Since this species is the most numerous of all kinds of furcocercous cercariae found in Douglas Lake (Cort and Brooks, 1928, p. 183), it probably is also a proportionally important cause of the death of fishes there.

My experiments, those of Blochmann (1910, pp. 47-49), and of Cort and Brown¹ have shown that strigeid cercariae will penetrate and may kill other fishes than those in which they normally develop. Consequently, the deaths not only of the common suckers of Douglas Lake, but also of other fishes may be due to the

¹ Professor W. W. Cort and Dr. H. W. Brown have kindly permitted me to publish their records of the results of infestation experiments with cercariae of *D. flexicaudum* upon three kinds of fishes and also upon frog tadpoles. The fishes employed in these experiments were obtained from Vincent Lake, an acid lake (Jewell and Brown, 1924, pp. 77-84) in which the fishes were known to be free from trematode infestations. Fourteen yellow perch, *Perca flavescens*, and about one hundred young bull-heads, *Ameiurus nebulosus*, were exposed to these cercariae. About one month later the fishes of both species which still survived contained variable numbers of not yet fully developed metacercariae in the lenses of their eyes. A similar exposure of two small pickerel, *Esox lucius*, yielded, six weeks later, fully developed specimens of diplostomulum in their crystalline lenses. Other experiments proved that these cercariae readily penetrated other kinds of fishes. Tadpoles exposed similarly contained only degenerating cercariae, also in their eyes, after one month.

cercariae of *D. flexicaudum*. Likewise, the deaths of the common suckers of Douglas Lake are doubtless due not to this species of cercaria alone, but also to other strigeid cercariae. On this account it appears that these fatal epidemics among fishes cannot be prevented by the destruction of a few species of snails, since many kinds of snails and cercariae are involved in the deaths. Though a reduction of the number of Lymneidae in a lake, as Szidat (1927, pp. 83-90) proposed, will help to reduce the mortality rate of fishes, it is hoped that a careful study of the physiological requirements of miracidia and cercariae will suggest more practicable measures for the conservation of our fishes.

As already explained in the introductory description of the life-history of this species, there are often found in nature common suckers which have large numbers of fully developed diplostomula of *D. flexicaudum* in the lenses of their eyes. Usually these fishes do not show marked symptoms which can be attributed to their infestations. The infestation has doubtless taken place so gradually that the injuries inflicted by the penetrating cercariae were not fatal. Fatal injuries are inflicted only when large numbers of cercariae penetrate the fishes at the same time. After the metacercariae have lodged in the crystalline lenses of the eyes they probably are not severely injurious unless they are present in large numbers (Hughes and Berkhout, 1929, p. 484). Thus it can be explained that the common suckers of Douglas Lake may harbor thirty metacercariae of *D. flexicaudum* in the lens of each eye without succumbing to the infestation.

THE DIPLOSTOMULUM

Host and habitat. — As explained in the introductory description of my feeding experiments, an average of thirty diplostomula of *D. flexicaudum* was found in 94 per cent of the common suckers of Douglas Lake by La Rue, Butler and Berkhout (1926, pp. 284-286). They were found in the lenses and not in the humors of the eyes. These metacercariae are not encysted and they appear to be all of one kind. Their morphology has been de-

scribed by Hughes and Berkhout (1929, pp. 483-488) and therefore is not discussed here. In this connection it is significant to note that the metacercariae of *Diplostomum spathaceum* (Rud.), called *Diplostomum volvens* von Nordmann by Braun (1894, pp. 165-167) in the description of the feeding experiments performed by the Ehrhardt brothers and also by Szidat (1927, pp. 83-90) in the description of the life-history of this species, was found in both the lenses and the humors of the eyes of *Acerina cernua* and *Leuciscus rutilus*. But the fully developed diplostomula of *D. flexicauda* were found only in the lenses of the eyes of the common suckers, *Catostomus commersonnii*, the only species of fishes in which I have studied them. Because of the morphological differences described by Hughes and Berkhout (1929, pp. 483-488), it appears justifiable to distinguish specifically between the diplostomulum of *D. spathaceum* and that of *D. flexicaudum*. The belief that they are distinct species is also strengthened by my comparative study of the adults, presented in Part I of this paper.

Summary of life-history. — The rate of development of *D. flexicaudum* doubtless varies with the temperature, but at summer temperatures in Michigan development proceeds approximately as indicated in the following outline.

Eggs in the feces of the herring-gull are deposited in water. From these eggs miracidia hatch in about three weeks. After escaping from the eggs the miracidia penetrate *Lymnea emarginata angulata* Sowerby, and probably other snails, within a few hours. Inside the snails the miracidia transform into mother sporocysts which in turn produce daughter sporocysts. Within the latter cercariae develop in about six weeks after the penetration of the miracidium into the snail. The cercariae escape from the snails and lead a free-swimming life for less than a day. They penetrate various parts of the body of the common sucker and travel to the eyes of these fishes, finally coming to rest within the lens capsule, where they grow and metamorphose into the diplostomulum stage. It probably takes about five or six weeks from the time that the cercariae penetrate into the fishes before the diplostomulum has reached the infective stage. When common suckers are eaten by herring-gulls the diplostomulum attains sexual maturity in

their intestines in less than one week. The length of life of the adult *D. flexicaudum* within the gull was not determined.

The life-cycle of *D. flexicaudum* agrees in general with the description of the life-cycle of *D. spathaceum* as described by Szidat (1927, pp. 85-86), but Szidat does not explain the time required for the development of each stage. The snail and fish hosts of the two species differ. The rate of development of *D. flexicaudum* appears to agree quite closely, according to results in this paper (also Van Haitisma, 1931), with that of *Cotylurus flabelliformis* (Faust) and *C. michiganensis* (La Rue) (Van Haitisma, 1930), and also with that of *Strigea tarda* described by Mathias (1925).

The miracidium and the sporocyst have been carefully described, the former in considerable detail. The cercaria has been described by Cort and Brooks (1928, pp. 183-186) and the diplostomulum by Hughes and Berkhout (1929, pp. 483-486). Description of the adults and a comparison of adults with related forms has been made in Part I of this paper.

The economic importance of the cercaria is considered. Studies of the results of infestation experiments of the cercariae in fishes are discussed. They indicate clearly that the penetration of large numbers of these cercariae into fishes causes the death of the fishes. The conclusion is drawn that the cercariae of *D. flexicaudum* also cause the death of large numbers of fishes in breeding ponds and in nature.

ACKNOWLEDGMENTS

This study was conducted under the direction of Professor George R. La Rue, whose helpful suggestions have been invaluable. Professor W. W. Cort confirmed the identification of the cercaria. The fishes and snails employed in the experiments were identified by Dr. Carl Hubbs and Miss Mina L. Winslow, respectively. For all of this help, generously given, I wish to express my cordial gratitude.

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LITERATURE CITED

- BARLOW, C. H. 1925. The Life-Cycle of the Human Intestinal Fluke, *Fasciolopsis buski* (Lankester). Am. Journ. Hyg., Mon. Ser., No. 4, pp. 1-98, 10 pls., 12 text figs.
- BLOCHMANN, F. 1910. Sterben von Aquarienfischen durch Einwanderung von *Cercaria fissicauda* La Val. Centralbl. f. Bakt. . . , I Abt., Orig., 56 (Heft 11): 47-49.
- BRANDES, G. 1890. Die Familie der Holostomiden. Zool. Jahrb., Jena, Abt. f. Syst., 5: 549-604, 3 pls.
- 1892. Revision der Monostomiden. Centralbl. f. Bakt. . . , Jena, 12 (No. 15, 7 Okt.): 504-511.
- BRAUN, M. 1894. Zur Entwicklungsgeschichte der Holostomiden. Zool. Anz., 17: 165-167.
- COE, W. R. 1896. Notizen über den Bau des Embryos von *Distomum hepaticum*. Zool. Jahrb. Anat., 9: 561-570, 1 pl.
- CORT, W. W. 1919. Notes on the Eggs and Miracidia of the Human Schistosomes. Univ. Cal. Publ. Zool., 18: 509-519, 7 text figs.
- AND BROOKS, S. T. 1928. Studies on the Holostome Cercariae from Douglas Lake, Michigan. Trans. Am. Microsc. Soc., 47: 179-221, 5 pls., 6 text figs.
- DIESING, C. M. 1850. Systema helminthum. Bd. 1, Vindobonae.
- FAUST, E. C., AND MELENEY, H. E. 1924. Studies on *Schistosomiasis japonica*. Am. Journ. Hyg., Mon. Ser., No. 3, pp. 1-326, 26 pls.
- GUBERLET, J. E. 1922. Three New Species of Holostomidae. Journ. Parasitol., 9: 6-14, 2 pls.
- HUGHES, R. C., AND BERKHOUT, P. G. 1929. Studies on the Trematode Family Strigeidae (Holostomidae). No. XV. *Diplostomulum gigas*, Sp. Nov. Pap. Mich. Acad. Sci., Arts and Letters, 10: 483-488, 2 pls.
- HUGHES, R. C., AND HALL, L. J. 1929. Studies on the Trematode Family Strigeidae (Holostomidae). No. XVI. *Diplostomulum huronense* (La Rue). *Ibid.*, 10: 489-494, 2 pls.
- JEWELL, M. E., AND BROWN, H. W. 1924. The Fishes of an Acid Lake. Trans. Am. Microsc. Soc., 43: 77-84, 2 text figs.
- KRAUSE, R. 1915. Beitrag zur Kenntnis der Hemistominen. Zeitschr. f. wissensch. Zool., 112: 93-238, 1 pl., 78 text figs.
- LA RUE, G. R. 1926. Studies on the Trematode Family Strigeidae (Holostomidae). No. III. Relationships. Trans. Am. Microsc. Soc., 45: 265-280, 1 pl.
- 1927. Studies on the Trematode Family Strigeidae (Holostomidae). No. V. *Proalaria huronensis*, Sp. Nov. *Ibid.*, 46: 26-35, 2 pls.

- BUTLER, E. P., AND BERKHOUT, P. G. 1926. Studies on the Trematode Family Strigeidae (Holostomidae). No. IV. The Eyes of Fishes an Important Habitat for Larval Strigeidae. *Ibid.*, 45: 282-288.
- MATHIAS, P. 1925. Recherches expérimentales sur le cycle évolutif de quelques trematodes. *Bull. Biol. France et Belg.*, 59: 1-123, 4 pls., 13 figs.
- ORTMANN, W. 1908. Zur Embryonalentwicklung des Leberegels (*Fasciola hepatica*). *Zool. Jahrb. Anat.*, 26: 255-292, 3 pls.
- POIRIER, J. 1886. Sur les Diplostomidae. *Arch. de zool. exp. et gen.*, 4: 327-346, 3 pls.
- SCHUBMAN, W. 1905. Über die Eibildung und Embryonalentwicklung von *Fasciola hepatica* L. (*Distomum hepaticum* Retz.). *Zool. Jahrb. Anat.*, 21: 571-606, 2 pls.
- SEWELL, R. B. S. 1922. Cercariae Indicae. *Ind. Journ. Med. Res. (Suppl.)*, 10: 1-370, 32 pls., 7 text figs.
- SSINITZIN, D. TH. 1909. Studien über die Phylogenie der Trematoden. I. Können die digenetischen Trematoden sich auf ungeschlechtlichem Wege fortpflanzen? *Biol. Centralbl.*, 29 (No. 21): 664-682, 1 pl., 1 fig.
- STILES, CH. W., AND HASSALL, A. 1908. Index Catalogue of Medical and Veterinary Zoology. Trematoda and Trematode Diseases. *Hyg. Lab. Bull. No. 37*. Washington, 401 pp.
- SZIDAT, I. 1924. Beiträge zur Entwicklungsgeschichte der Holostomiden. II. *Zool. Anz.*, 61: 249-266, 12 text figs.
- 1927. Über ein Fischsterben im Kurischen Haff und seine Ursachen. *Zeitschr. f. Fisch. u. deren Hilfswiss.*, 25: 83-90, 5 text figs.
- THOMAS, A. P. 1882. The Life History of the Liver-Fluke (*Fasciola hepatica*). *Quart. Journ. Microsc. Sci., New Ser.*, 23: 99-133, 2 pls.
- VAN HAITSMA, J. P. 1925. *Crassiphiala bulboglossa* Nov. Gen., Nov. Spec., a Holostomatid Trematode from the Belted Kingfisher, *Ceryle alcyon* Linn. *Trans. Am. Microsc. Soc.*, 44: 121-131, 2 pls.
- 1930. Studies on the Trematode Family Strigeidae (Holostomidae). No. XXI. Life-Cycle and Description of *Cercaria michiganensis* (La Rue). *Journ. Parasitol.*, 16: 229-230.
- 1931. Studies on the Trematode Family Strigeidae (Holostomidae). No. XXII. *Cotylurus flabelliformis* (Faust) and Its Life-History. *Pap. Mich. Acad. Sci., Arts and Letters*, 13: 447-482.
- VON LINSTOW, O. 1877. *Enthelminthologica*. *Arch. f. Naturgesch.*, 43, Bd. 1, No. 2. Berlin.
- VON NORDMANN, A. 1832. *Mikrographische Beiträge zur Naturgeschichte der wirbellosen Tiere*. 1. Heft, Berlin.

EXPLANATION OF PLATES

The reference lines near the figures represent 0.1 mm.

ABBREVIATIONS

<i>ac</i> — acetabulum	<i>mg</i> — Mehlis' gland
<i>ag</i> — adhesive gland	<i>mvex</i> — median ventral excretory vessel
<i>bp</i> — birth-pore	<i>n</i> — nerve strand
<i>ct</i> — collecting tubule	<i>ns</i> — nervous system
<i>cut</i> — cuticle	<i>oot</i> — oötype
<i>cvd</i> — common vitelline duct	<i>ov</i> — ovary
<i>dc</i> — digestive cecum	<i>ovd</i> — oviduct
<i>dej</i> — ductus ejaculatorius	<i>rvd</i> — right vitelline duct
<i>dex</i> — dorsal excretory vessel	<i>t₁</i> — anterior testis
<i>ds</i> — digestive organ	<i>t₂</i> — posterior testis
<i>egc</i> — egg cell	<i>ut</i> — uterus
<i>ep</i> — epidermal plate	<i>uta</i> — uterus ascendens
<i>epl</i> — epidermal layer	<i>utd</i> — uterus descendens
<i>ex</i> — excretory vessel	<i>vd</i> — vas deferens
<i>exp</i> — excretory pore	<i>ve₁</i> — vas efferens of <i>t₁</i>
<i>ga</i> — genital atrium	<i>ve₂</i> — vas efferens of <i>t₂</i>
<i>gb</i> — germ ball	<i>ves</i> — vesicle
<i>gc</i> — germ cell	<i>vex</i> — ventral excretory vessel
<i>l</i> — lens	<i>vit</i> — vitelline follicle
<i>lc</i> — Laurer's canal	<i>vr</i> — vitelline reservoir
<i>lp</i> — lateral process	<i>vs</i> — vesicula seminalis
<i>lsc</i> — lateral sucking cup	

EXPLANATION OF PLATES XLIII-XLV

PLATE XLIII

- FIG. 1. Reconstruction showing external form, distribution of vitellaria and digestive system of *D. flexicaudum*. Right side view
- FIG. 2. Reconstruction of male reproductive system of *D. flexicaudum*. Right side view
- FIG. 3. Reconstruction of cross-sections, showing the coils of the seminal vesicle of *D. flexicaudum*
- FIG. 4. Reconstruction of cross-sections of *D. flexicaudum*, showing the relation of the ejaculatory duct and the uterus to the genital atrium
- FIG. 5. Reconstruction of cross-sections of *D. flexicaudum*, showing the relation of the vasa efferentia to the vas deferens and anterior testis
- FIG. 6. Reconstruction of cross-sections, showing the relation of the vitelline reservoir, common vitelline duct, oötype, Mehlis' gland and ascending uterus. Anterior view

- FIG. 7. Reconstruction showing the relation of the ascending uterus to the ovary, oviduct, and Laurer's canal. Anterior view
- FIG. 8. Right side view of the ovary and the junction of Laurer's canal with the oviduct
- FIG. 9. Right side view of the intertesticular coils of the uterus
- FIG. 10. Right side view of the female genital system

PLATE XLIV

- FIGS. 11-19. A series of cross-sections of an early adult developmental stage to show the excretory system
- FIG. 11. Cross-section of the forebody anterior to the acetabulum
- FIG. 12. Cross-section of the forebody in the region of the holdfast organ
- FIG. 13. Cross-section of the posterior end of the forebody
- FIG. 14. Cross-section of the hindbody at the level of the anterior bend in the uterus
- FIG. 15. Cross-section of the hindbody through Laurer's canal
- FIG. 16. Cross-section of the hindbody through the anterior testis
- FIG. 17. Cross-section of the hindbody through the posterior testis
- FIG. 18. Cross-section of the hindbody through the bursa copulatrix
- FIG. 19. Cross-section of the hindbody through the excretory pore

PLATE XLV

- FIG. 20. Epidermal plates of the miracidium of *D. flexicaudum*
- FIG. 21. Optical view of epidermal plates and lateral processes of the miracidium of *D. flexicaudum*
- FIG. 22. Relation of larger lateral process to vesicle in the miracidium of *D. flexicaudum*. A slightly deeper focus than shown in Figure 21
- FIG. 23. Optical view of excretory pore of the miracidium of *D. flexicaudum*
- FIG. 24. A reconstruction of many sketches of the internal organs of the miracidium of *D. flexicaudum*
- FIG. 25. A longitudinal section of the anterior end of the sporocyst of *D. flexicaudum*, showing the birth-pore
- FIG. 26. An external view of the posterior end of the sporocyst of *D. flexicaudum* showing egg cells and germ balls

PLATE XLIII



PLATE XLIV

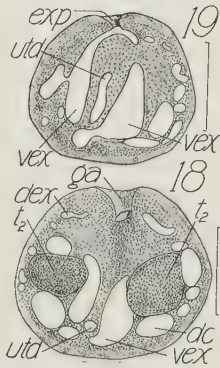
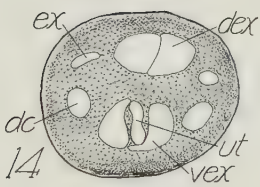
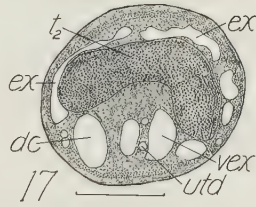
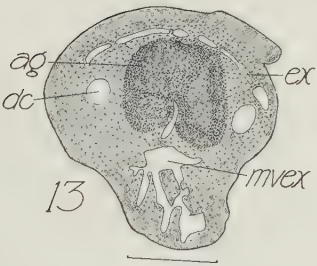
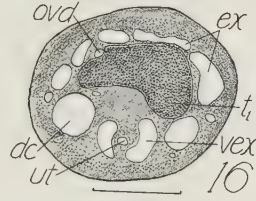
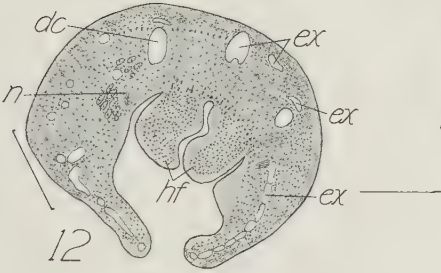
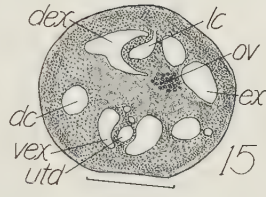
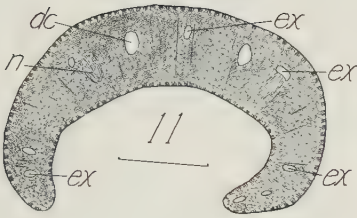
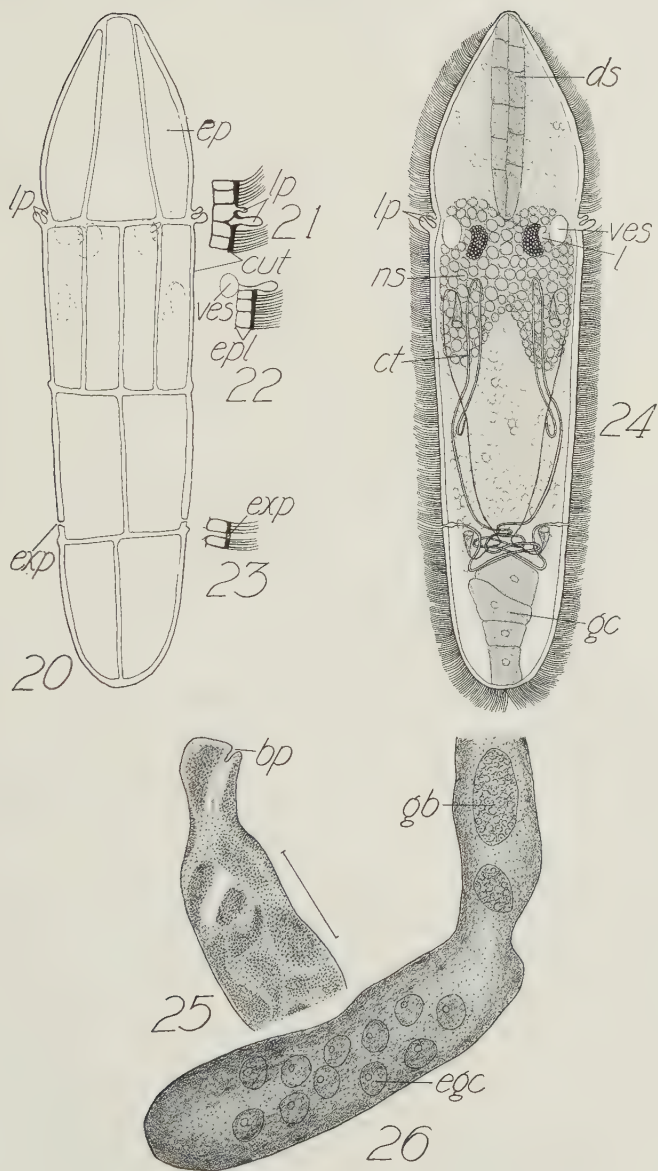


PLATE XLV



STUDIES ON THE TREMATODE FAMILY STRIGEIDAE (HOLOSTO-
MIDAE) NO. XX. *PARADIPLOSTOMUM PTYCHOCHEILUS*
(FAUST)

J. P. VAN HAITSMAN

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STUDIES ON THE TREMATODE FAMILY STRIGEIDAE
(HOLOSTOMIDAE)

No. XX. *PARADIPLOSTOMUM PTYCHOCHEILUS* (FAUST)*

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A new adult trematode of the family Strigeidae, here described as *Paradiplostomum ptychocheilus* (Faust), was found in four species of ducks. This parasite inhabits hosts differing widely from the hosts of other known species of this genus and it differs markedly in several structural characters from other known species of strigeids from ducks. Some of its organs have an unusual arrangement.

Material. The specimens were obtained August 17, 1923, from the intestine of an American merganser, *Mergus americanus* (Cass.) shot on Douglas Lake, Cheboygan county, Michigan. Later, similar parasites were obtained from intestines kindly sent to me by Mr. Earl Lambert who also identified the hosts: red breasted merganser, *Mergus serrator* (Linn.), hooded merganser, *Lophodytes cucullatus* (Linn.), and the old squaw duck, *Harelda hyemalis* (Linn.), all collected near Carthage, Illinois.

Specific diagnosis. *Paradiplostomum*: forebody, thin, broadly rounded anteriorly, concave ventrally, twice as long as the rounded conical hindbody from which it is not distinctly set off by a groove; total length, 0.47-0.53 mm., breadth at acetabulum, 0.23-0.28 mm.; oral sucker nearly circular, 0.025-0.03 mm. in diameter; acetabulum circular, 0.03 mm. in diameter; holdfast organ when protruded about 0.10 mm. in diameter; bursa copulatrix, large, protrusible, bell-shaped; eggs few, usually only one or two, 0.07-0.075 by 0.06-0.065 mm.; excretory bladder, large, lining nearly all of the dorsal wall of the hindbody; anterior testis, flattened ellipsoidal, diagonal in position, asymmetrically placed in the right half of the hindbody; posterior testis more symmetrically placed, bilobed, right and left lobes connected by a thin mid-piece; seminal vesicle large, about 0.10 mm. long and 0.05 mm. in diameter; ovary, ellipsoidal, 0.04-0.05 by 0.06-0.07 mm.; Mehlis' gland, pyramidal, on the left side of the hindbody, not between the testes; ascending limb of the uterus very short; descending limb of the uterus lying between the seminal vesicle and the posterior testis; habitat, intestine of fish ducks.

Size and external features. Individuals of this species are so small and

* Contribution from the Biological Laboratory of Calvin College and from the Biological Station and the Zoological Laboratory of the University of Michigan. This is the twentieth of a series of studies on the family Strigeidae by Professor George R. LaRue, his students and associates.

look so much like strigeid metacercariae (Figs. 4 and 5), that they were at first mistaken for immature forms. Closer examination, however, revealed that generally they contained one or two large eggs and that the reproductive organs were fully developed. More than one hundred of these parasites, of about the same stage of development, were found in one bird. Consequently, the conclusion seemed justified that these parasites had attained full development. Measurements showed this to be one of the smallest species of the family Strigeidae. The largest specimens measure scarcely more than one-half millimeter long and one-fourth millimeter broad. Both anterior and posterior ends are broadly rounded. Although the body is not marked off by a distinct groove, two body regions may be distinguished readily since the forebody is comparatively thin and concave ventrally while the hindbody is short and bluntly conical and rounded out in all directions. The forebody is somewhat wider and fully twice as long as the hindbody.

The oral sucker is nearly circular and terminal (Fig. 13), but it often appears to have a subterminal position because the anterior end of the forebody is usually inturned ventrally (Figs. 4 and 5). The acetabulum, nearly equal to the oral sucker in size, is found in the posterior third of the forebody. The holdfast organ may be found either retracted or protruded. When retracted, its tissues are arranged so as to form a deep cavity with a variously shaped opening surrounded by a slightly projecting ridge (Fig. 4); when protruded, it appears as a hollow truncated cone whose base covers the acetabulum (Figs. 5 and 6). The genital pore is in the usual position. From the pore a large bell-shaped bursa copulatrix often protrudes (Figs. 2, 9, 13). Inside of the bursa of some specimens a long genital cone projects outward from the body almost to the opening of the bursa.

Type specimen and paratypes. The type specimen of this species (Figs. 3, 5, and 7) is 0.49 mm. long and 0.28 mm. wide at the level of the acetabulum. It is labelled S-Z-2 in the author's collection. Paratypes of this species have been placed in the University of Michigan Museum of Zoology where it is recorded as number 201 and in the United States National Museum under the number 7990. The habitat of the type and paratypes was the intestine of *Mergus americanus* (Cass.) obtained August 17, 1923, at Douglas Lake, Cheboygan County, Michigan.

Table I. Measurements in millimeters of six specimens of *Paradiplostomum ptychocheilus* (Faust)

Items	Range	Mean	Type-specimen
Total length	0.47-0.53	0.487	0.49
Total breadth (at acetabulum)	0.23-0.28	0.247	0.28
Oral sucker,			
length	0.025-0.03	0.029	0.03
breadth	0.025-0.03	0.027	0.025

Pharynx,			
length	0.03-0.035	0.031	0.03
breadth	0.02-0.025	0.024	0.025
Acetabulum,			
diameter	0.03	0.03	0.03
Anterior testis,			
length (anteropost.)	0.07-0.085	0.074	0.085
breadth	0.09-0.12	0.104	0.12
Posterior testis,			
breadth	0.17-0.20	0.183	0.19
Ovary,			
length (anteropost.)	0.04-0.05	0.043	0.04
breadth	0.06-0.07	0.0675	0.065
Uterine egg			
length	0.07-0.075	0.071	0.075
breadth	0.06-0.065	0.061	0.065

Measurements. Measurements of six specimens and of certain of their organs are recorded in table I. These measurements are compared with corresponding measurements of the type specimen. The variations in body dimensions are due partly to the variable infolding of the anterior end and lateral margins of the forebody and partly to the different contraction states of the specimens. All of the specimens measured were sexually mature. The thickness of the forebody dorsoventrally at the level of the acetabulum is 0.085 mm. (two measurements). The forebody becomes gradually thinner anteriorly. The hindbody at the level of the exterior opening of Laurer's canal is 0.16 mm. thick dorsoventrally (two measurements), but it thickens anteriorly and thins posteriorly.

Subcuticular glands. Immediately within the outer muscular layer of both dorsal and ventral surfaces of the forebody occur rounded vesicles containing a glandular secretion. These vesicles (Fig. 11) are arranged in rows between the longitudinal muscles. They appear to be connected with processes of deeper-lying glandular cells, but the connections could not be traced satisfactorily (Fig. 10). Krause (1915:106) carefully described these glands (Hautdrüsen) for three species of hemistomes.

Holdfast organ. When protruded the holdfast organ (Figs. 5 and 6) is a truncated cone whose base, 0.10 mm. in diameter (two measurements), projects from the ventral surface. Its narrow and deep cavity is a longitudinal slit. In the retracted condition (Figs. 4 and 13) of the holdfast organ the opening is elongated longitudinally, but its cavity, extending deeply into the tissues, becomes wider in the transverse direction and narrower in the antero-posterior direction.

Adhesive gland. The adhesive gland (Figs. 5, 6, and 13), roughly C-shaped in both frontal and sagittal sections, surrounds the cavity of the holdfast organ. It is composed of elongated ovoid cells some of whose proc-

esses (Fig. 13) could be traced as far as the cuticula of the holdfast organ. This gland is less compact in the present species than in some other members of the family. Its cells are arranged in definite rows around the inner end of the cavity of the holdfast organ.

Digestive system. With respect to this system the present species resembles other members of the family. The prepharynx (Fig. 4) is short, oral sucker and pharynx are nearly equal in size. The esophagus measures 0.02-0.04 mm. long. The unbranched digestive ceca extend to the middle region of the hindbody (Figs. 5 and 8) where they become enlarged and bend toward the median line. In every individual examined they appeared to be empty.

Excretory system. The excretory system (Figs. 5 and 6) occupies a large part of the peripheral region of the hindbody. The excretory pore is postero-terminal. From it the short common excretory duct extends anteriorad and receives a pair of short wide lateral excretory vessels. These vessels represent the right and left lobes of the excretory bladder, each of which gives rise to a dorsal and a ventral vessel. The two dorsal vessels extend along the sides of the hindbody, but are so wide that they also line nearly all of the dorsal wall of the hindbody. At the anterior end of the hindbody they unite into one median dorsal vessel. The two ventral vessels, one from each lobe of the bladder, are smaller than the dorsal vessels. They extend anteriorad along the ventral surface into the region containing vitelline follicles where they become small and difficult to trace.

In the forebody some of the more conspicuous excretory canals were also traced (Fig. 12), but they have not been connected with the excretory vessels of the hindbody. A median excretory canal extends posteriorad from the pharynx dorsal to the acetabulum. It probably joins the median dorsal excretory vessel of the hindbody. In the region of the pharynx the median excretory canal divides into two branches, one on each side of the pharynx. From each of these side branches three main longitudinal canals were traced posteriorad. The innermost of these three canals is located near the digestive cecum, the middle one extends along the nerve strand, while the third longitudinal canal lies nearer the lateral margin of the forebody. Many transverse canals connect these three longitudinal canals into an irregular network (Fig. 12).

Male genital system. The male sexual system (Figs. 7, 8, and 9) consists of the usual organs. The organs of this system are extraordinarily large compared with the size of the hindbody. The anterior testis, flattened ellipsoidal in form, occurs on the right side in the anterior part of the hindbody. In a posterior view it occupies a diagonal position (Fig. 8) since its greatest diameter extends from the median dorsal to the mid-lateral region of the hindbody. This unusual position of the anterior testis is ascribed to the

pressure of the large egg often found in the uterus immediately ventral to the median end of this testis. In some specimens the left half of the anterior testis appeared to be distinctly flattened by the pressure of the egg. This flattening also accounts for the difference in measurements of the breadth of this testis in table I. The posterior testis overlaps the anterior one when viewed from the left side, the posterior end, or the ventral surface. It consists of thick right and left lobes connected by a much thinner middle piece. The left lobe is somewhat larger than the right. The general shape of this testis is that of a bean deeply concave at the hilum. Its greatest diameter extends nearly across the hindbody whose posterior half it largely occupies.

Either because the vasa efferentia do not contain a large number of spermatozoa or because they lie closely appressed to the thicker walled uterus, they were so difficult to trace that they are somewhat doubtfully described. In two specimens, however, one sectioned frontally and the other sagittally (Figs. 7, 9, and 13), the vasa efferentia appeared to arise midventrally near the anterior margins of their respective testes, the vas efferens of the anterior testis (ve_1) extending posteriad and mesad and the vas efferens of the posterior testis (ve_2) antieriad and dextrad to their junction with each other and with the vas deferens. The latter is short and empties ventrad and posteriad into the ellipsoidal seminal vesicle. This vesicle, filled with spermatozoa, is extraordinarily large for so small an animal. It is about 0.10 mm. long and 0.05 mm. in diameter. It lies in the hollow formed by the thin median region of the posterior testis and extends nearly to the posterior end of the animal. Near its posterior end and to the right of its mid-dorsal line it opens dorsad and dextrad into the thicker walled conoidal ejaculatory pouch. The transverse diameter of this pouch is about 0.04 mm. The anterior dorso-median end of this pouch is continuous with the thick-walled ejaculatory duct. This duct arches posteriad and mesad to empty through the genital cone into the genital atrium, the cavity of the bursa copulatrix. The genital cone opens to the outside through the bursa copulatrix on the posterior mid-dorsal surface of the hindbody.

Female genital system. The ovary and the Mehlis' gland (Figs. 2, 3, 8, and 13) lie in the left anterior region of the hindbody opposite the anterior testis. The ovary is ellipsoidal in form and is situated more dorsally and nearer the median plane. From its posterior dorsal side arises the oviduct which extends mesad and posteriad to its junction with Laurer's canal, then turning sinistrad and bending antieriad it enters the left dorsal region of the Mehlis' gland. Here the common vitelline duct joins the oviduct and then the oviduct enlarges and becomes the thicker-walled oötype. Bending abruptly ventrad the oötype extends through the Mehlis' gland from left to right and from its dorsal to its ventral side. Since the ascending limb of the

uterus is so short as to be practically negligible, the oötype virtually empties into the descending limb of the uterus which bends posteriad and dorsad to the left of the vas deferens and, extending between the posterior testis and the seminal vesicle, finally empties into the genital atrium ventral to the opening of the ejaculatory duct. The course of the descending uterus along the dorsal side of the seminal vesicle is different from that of most members of the family.

Laurer's canal (Figs. 2, 3, 8, and 13) extends from its junction with the oviduct first posteriad and dextrad around the median end of the anterior testis and then bends dorsad and anteriorad and opens to the outside in the median dorsal line of the hindbody at the level of the ovary. Near its junction with the oviduct it is dilated. This enlargement of Laurer's canal contains spermatozoa, suggesting that it serves as a seminal receptacle. In this respect *Paradiplostomum pychocheilus* (Faust) resembles *Crassiphiala bulboglossa mihi* (Van Haitsma, 1925:125-126).

The Mehlis' gland (Figs. 2, 3, and 8) is situated ventrally, posteriorly, and to the left of the ovary, opposite the anterior testis. It is roughly pyramidal in shape being triangular in outline when viewed from the posterior end, the ventral surface, or the left side. Its shape may be modified, however, by the extent of the dilation of the oötype and by the pressure of eggs in the uterus.

Vitelline system. Large, brass-colored, vitelline follicles (Figs. 4 and 5) occupy four lateral fields when the worm is seen in ventral view. On each side of the forebody two of these lateral fields extend forward somewhat beyond the acetabulum while the other two lateral fields extend backward in the hindbody on each side of the seminal vesicle. Wide right and left vitelline ducts extend dorsad on the right and the left sides of the bend in the uterus in the anterior part of the hindbody and discharge into the vitelline reservoir (Figs. 8 and 13). The latter is surrounded by the genital glands, the ovary and anterior testis lying in front of it and the posterior testis behind it. The larger part of this reservoir lies on the left side of the median sagittal plane. It is an ellipsoidal sac about 0.10 mm. in length and 0.06 mm. in diameter. This reservoir empties on the left into the common vitelline duct which extends through the posterior dorsal side of the Mehlis' gland and bending anteriorad joins the oviduct where this enters the Mehlis' gland, after which the oviduct becomes the oötype.

Displacement of organs. Associated with the fact that the hindbody is very short and broad many of the organs of this region occupy positions different from those in most Strigeidae. The positions of the anterior testis, the ovary, and the Mehlis' gland are such as would be produced by shifting the anterior testis dorsad and to the right, pushing the ovary backward and to the left, and drawing the Mehlis' gland forward and to the left. The

vitelline reservoir also appears to be shifted somewhat to the left. In consequence of the apparent displacement of these organs the oviduct does not extend completely around the anterior testis, but only around its left end. Consequently also the larger part of the course of the oviduct is mainly transverse rather than anteroposterior as in most Strigeidae.

Bursa copulatrix. The bursa copulatrix may be either in a retracted or in an extended condition. When retracted (Figs. 3 and 7) its opening forms a narrow longitudinal slit near the posterior end of the dorsal body wall. When extended (Figs. 2, 9, and 13) the bursa copulatrix is a large, short, bell-shaped, muscular protrusion with a nearly circular opening. It contains a C-shaped mass of muscles, deeply indented ventrally and open dorsally. A thick genital cone extends anteroposteriad through the cavity of the bursa to the outside. Into the genital atrium the ejaculatory duct and uterus open close together, dorsal and ventral to each other (Figs. 8 and 13). The bursa copulatrix and the genital cone are so constructed that it appears that they might serve as copulatory organs. Direct observation of copulation is very rare in this family (Brandes, 1890:566-567).

Egg. In the uterus in the anterior midventral part of the hindbody one or two eggs are generally present. Although these eggs are smaller (0.07-0.075 by 0.06-0.065 mm.) than those of other known Strigeidae, they are very large when compared with the size of the hindbody of the animal. Each egg is almost twice as large as the ovary. The passage of the eggs through the uterus must cause great changes in shape and position of the organs of the hindbody.

Metacercaria. The similarity in form and structure of my adult specimens and the metacercaria, *Neascus pychocheilus* (Faust, 1917:110 and 1917a:66) Hughes and Piszczek (1928: 58-62, especially plate 6) suggested that the adult of my species might have developed from this metacercaria which is found in large numbers in cysts in the peritoneum and mesentery of the straw-colored minnow, *Notropis deliciosus stramineus* Cope, at Douglas Lake. No other *Neascus* occurs in this minnow in this lake. The specific identity of this metacercaria and my material was indicated by their similarity in outline, in the lateral excretory network of the forebody, in the extensive excretory bladder of the hindbody, and in the absence of a postero-ventral lip of the forebody.

To test this hypothesis by a feeding experiment it was necessary to substitute domestic ducks for fish ducks since young fish ducks were not available. During the summer of 1929 two young domestic ducks, whose feces proved to be free from strigeid eggs, were fed twenty-five straw-colored minnows which contained the above named metacercariae. Three days later a postmortem examination of the intestines of these birds revealed that they were infested with about one hundred parasites similar in all respects to my material ob-

tained from the fish ducks. This feeding experiment, therefore, clearly indicated the correctness of my hypothesis that *Neascus ptychocheilus* (Faust) is the metacercaria of my adults. Consequently the present species is *ptychocheilus*, but the genus to which this species belongs still requires consideration.

Classification. The presence of the large holdfast organ on the ventral surface behind the acetabulum together with the genital pore on the dorsal surface near the posterior end indicates clearly that the trematode here described belongs to the family Strigeidae Railliet. The flattened forebody, the circular holdfast organ, the absence of cirrus and cirrus pouch, with the uterus confined to the hindbody, characterizes the present parasite and places it in the subfamily Polycotylinæ Monticelli.

The attempt to determine the proper genus of the present species led into a taxonomic tangle which requires resolution before further classification is possible. In 1925 Poche (1925:190-192) erected the genus *Crocodilicola* with the type species, *C. pseudostoma* (Will.-Suhm), but Poche's diagnosis of this genus is so indefinite that it does not indicate a recognizable group of species. His diagnosis is impracticable. For this reason I propose to emend it in conformity with its type species so that it shall read as follows:

Genus *Crocodilicola* Poche 1925

Generic diagnosis. Forebody not distinctly set off from the hindbody. No lateral suckorial cups or ear-like appendages. No sucker-like formations on the dorsal side. Ovary close to and in front of the anterior testis. Oviduct rather long. Mehlis' gland and vitelline reservoir between the testes.

Type species.—*Crocodilicola pseudostoma* (Will.-Suhm).

Emended in this way the diagnosis of *Crocodilicola* describes a recognizable group of species by means of characters that readily distinguish it from other genera of its subfamily. In my species the Mehlis' gland is not situated between the testes and the oviduct is not remarkably long. Therefore this species cannot be included in the genus *Crocodilicola*.

The emended diagnosis of *Crocodilicola* clearly differentiates it from the genus *Paradiplostomum* LaRue 1926 which is a valid genus because its type species, *Paradiplostomum abbreviatum* (Brandes), is not congeneric with *C. pseudostoma* (Will.-Suhm). The present species resembles the type species of *Paradiplostomum*, *P. abbreviatum* (Brandes), more than any other known species of the Polycotylinæ and it is therefore included in the genus *Paradiplostomum*. Both the type species of this genus and the present species are characterized by a crowded condition of the organs of the hindbody. In both of these species the hindbody is considerably shorter than the forebody. In both, the ovary lies at the same level as the anterior testis and both have a large protruding bursa copulatrix. Brandes described a definite prostate gland in the genital cone of *P. abbreviatum*, but it is believed that his ob-

servation or interpretation may have been incorrect. In *P. ptychocheilus* the genital cone is not a solid conical organ as it is in *P. abbreviatum* (Brandes, 1890: plate XXXIX, fig. 16).

Summary. *Paradiplostomum ptychocheilus* is described as a new species of adult. Especially noteworthy are its small size, short hindbody, crowded condition of the reproductive organs involving an unusual position of the Mehlis' gland, relatively large eggs few in number, and the large bursa copulatrix. Feeding experiments demonstrated that this adult developed in the domestic duck from *Neascus ptychocheilus* (Faust) Hughes and Piszczek. The diagnosis of the genus *Crocodilicola* Poche 1925 has been emended.

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LITERATURE CITED

- Brandes, G. 1890.—Die Familie der Holostomiden. Zool. Jahrb., Jena, Abt. f. Syst., 5:549-604, pls. 39-41.
- Faust, E. C. 1917.—Notes on the cercariae of the Bitter Root Valley, Montana. Jour. Parasit., 3:105-123, 1 pl.
- 1917a. Life history studies on Montana trematodes. Ill. Biol. Monographs, 4:1-121, 9 pls.
- Hughes, R. C. and Piszczek, F. R. 1928.—Studies on the trematode family Strigeidae (Holostomidae). No. XI. *Neascus ptychocheilus* (Faust). Jour. Parasit., 15:58-62, 1 pl.
- LaRue, G. R. 1926.—Studies on the trematode family Strigeidae (Holostomidae). No. II. Taxonomy. Trans. Am. Microsc. Soc., 45:11-19.
- Poche, F. 1926.—Das System der Platyodaria. Arch. f. Naturg., Berlin (1925), Abt. A, Jan., 91:1-240, figs. 1-5, pls. 1-3, figs. 1-95; Mar., (3):241-458, figs. 7-16, pls. 4-7, figs. 96-126.
- Poirier, J. 1886.—Sur les Diplostomidae. Arch. de Zool. Exp. et Gén., 4:327-346, 3 pls.
- Van Haitsma, J. P. 1925.—*Crassiphiala bulboglossa* nov. gen., nov. spec., a holostomatid trematode from the belted kingfisher, *Ceryle alcyon* Linn. Trans. Am. Microsc. Soc., 44:121-131, 2 pls.

EXPLANATION OF FIGURES

The reference line near each figure represents 0.1 mm.

Abbreviations

ac	acetabulum	mg	Mehlis' gland
ag	adhesive gland	n	nerve strand
bc	bursa copulatrix	oot	oötype
cvd	common vitelline duct	os	oral sucker
eg	egg	ov	ovary
ejd	ejaculatory duct	ovd	oviduct
ejp	ejaculatory pouch	ph	pharynx
eso	esophagus	pph	prepharynx
ex	excretory bladder	rdex	right dorsal excretory vessel
exc	excretory canal	rvd	right vitelline duct
exp	excretory pore	sv	seminal vesicle
ga	genital atrium	t ₁	anterior testis
hf	holdfast organ	t ₂	posterior testis
int	digestive cecum	ut	uterus
lc	Laurer's canal	vd	vas deferens
ldex	left dorsal excretory vessel	ve ₁	vas efferens of t ₁
lvd	left vitelline duct	ve ₂	vas efferens of t ₂
lvex	left ventral excretory vessel	vf	vitelline follicle
mexc	median excretory canal	vr	vitelline reservoir

PLATE XV

- Fig. 1. Cross-section of the forebody at the level of the acetabulum.
Fig. 2. Reconstruction of female genital system, viewed from the left.
Fig. 3. Reconstruction of female genital system. Ventral aspect.
Fig. 4. Whole mount, stained and cleared. Ventral view.
Fig. 5. Reconstruction, ventral view, showing digestive, nervous, and excretory systems.

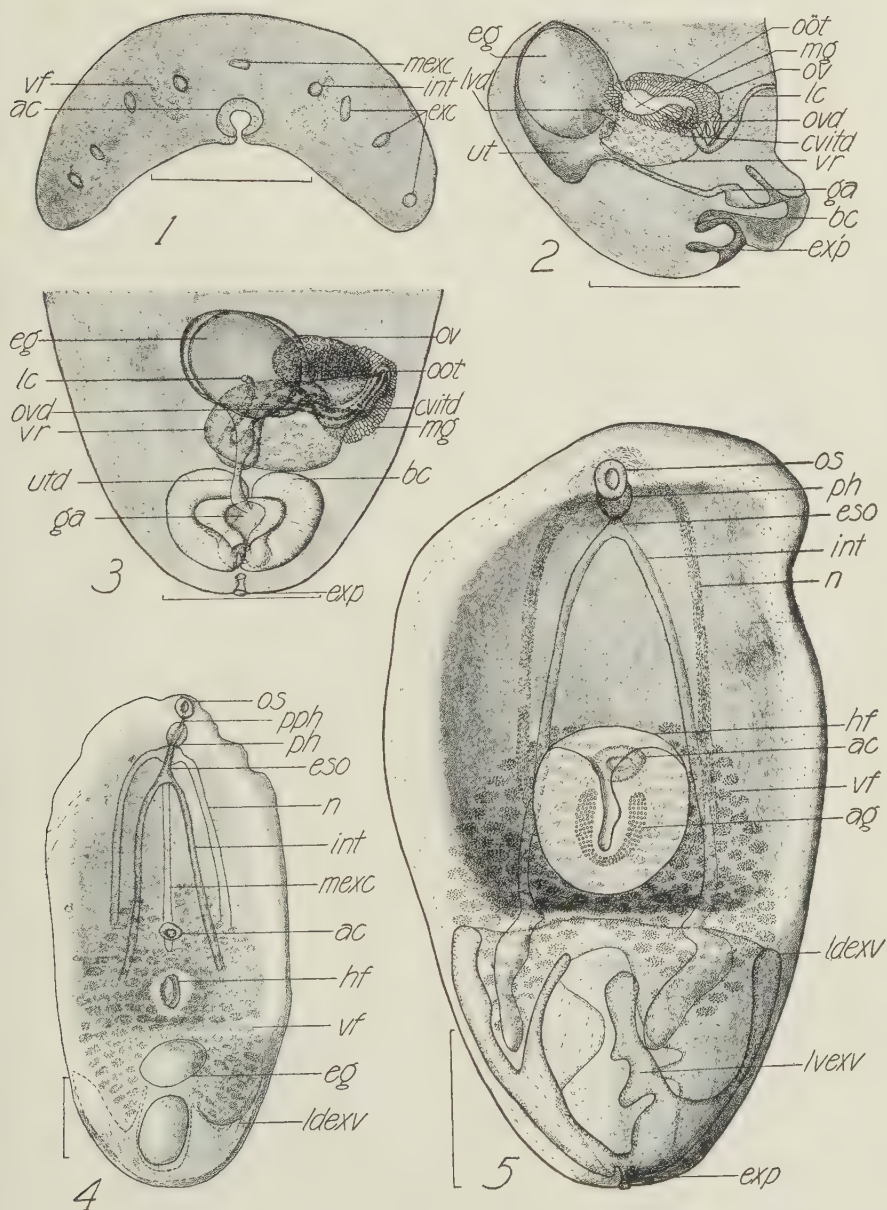


PLATE XV

PLATE XVI

- Fig. 6. Median sagittal section of the holdfast organ in the protruded condition.
- Fig. 7. Reconstruction, ventral view, of the male genital system.
- Fig. 8. Reconstruction of several cross-sections of the hindbody anterior and posterior to Laurer's canal. Posterior aspect. Vitellaria and dorsal excretory vessels not shown.
- Fig. 9. Reconstruction, left side view, of the male genital system.
- Fig. 10. Section of body wall showing subcuticular glands.
- Fig. 11. Subcuticular glands. Surface view.
- Fig. 12. Reconstruction of a few frontal sections of the anterior part of the forebody showing the distribution of the more conspicuous excretory canals.
- Fig. 13. Reconstruction of a few nearly median sagittal sections, viewed from the left side.

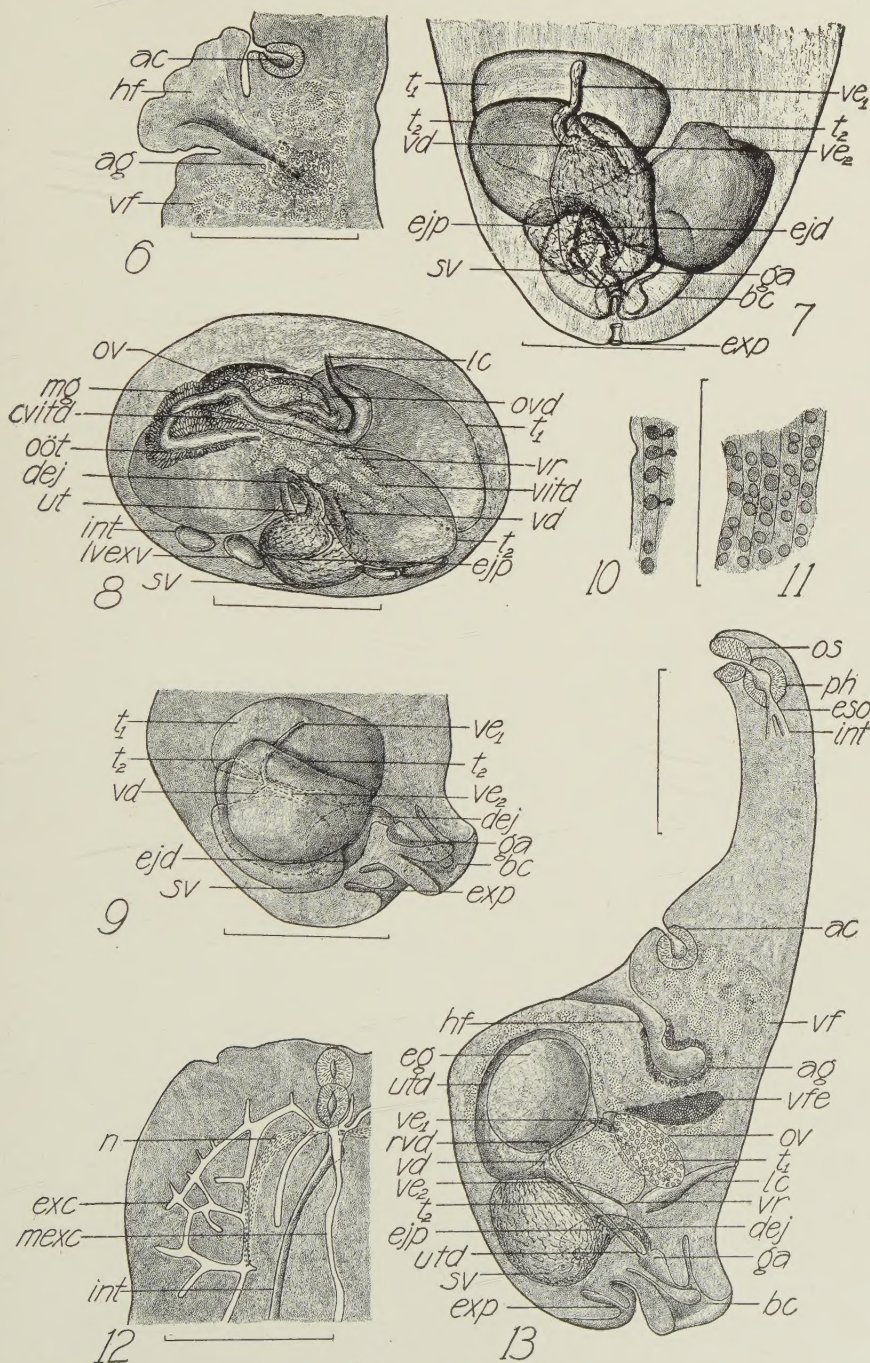


PLATE XVI

